08/876,276 Search Strategy/Results

(FILE 'HOME' ENTERED AT 08:22:03 ON 03 MAY 2002)

FILE 'CAPLUS' ENTERED AT 08:22:11 ON 03 MAY 2002
E ZHANG/AU 25

FILE 'MEDLINE, AGRICOLA, CAPLUS, BIOSIS, EMBASE, WPIDS' ENTERED AT 08:29:22 ON 03 MAY 2002

L1	149646	S FLOW (W) CYTOMETRY
L2	866	S L1 AND LIBRARY
L3	392	S L2 NOT PY>1997
L4	107	S L3 AND (FLUORESCENCE OR FLUORESCENT)
L5	66	DUP REM L4 (41 DUPLICATES REMOVED)

FILE

L5 ANSWER 1 OF 66 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 97405894 MEDLINE
DOCUMENT NUMBER: 97405894 PubMed ID: 9260938
TITLE: The glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase of Candida albicans is a surface antigen.
AUTHOR: Gil-Navarro I; Gil M L; Casanova M; O'Connor J E; Martinez J P; Gozalbo D
CORPORATE SOURCE: Departamento de Microbiologia y Ecologia, Facultad de Farmacia, Universitat de Valencia, Spain.
SOURCE: JOURNAL OF BACTERIOLOGY, (1997 Aug) 179 (16) 4992-9. Journal code: HH3; 2985120R. ISSN: 0021-9193.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-U72203
ENTRY MONTH: 199709
ENTRY DATE: Entered STN: 19970922
Last Updated on STN: 19990129
Entered Medline: 19970905

AB A lambda gt11 cDNA **library** from Candida albicans ATCC 26555 was screened by using pooled sera from two patients with systemic candidiasis and five neutropenic patients with high levels of anti-C. albicans immunoglobulin M antibodies. Seven clones were isolated from 60,000 recombinant phages. The most reactive one contained a 0.9-kb cDNA encoding a polypeptide immunoreactive only with sera from patients with systemic candidiasis. The whole gene was isolated from a genomic **library** by using the cDNA as a probe. The nucleotide sequence of the coding region showed homology (78 to 79%) to the Saccharomyces cerevisiae TDH1 to TDH3 genes coding for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and their amino acid sequences showed 76% identity; thus, this gene has been named C. albicans TDH1. A rabbit polyclonal antiserum against the purified cytosolic C. albicans GAPDH (polyclonal antibody [PAb] anti-CA-GAPDH) was used to identify the GAPDH in the beta-mercaptoethanol extracts containing cell wall moieties. Indirect immunofluorescence demonstrated the presence of GAPDH at the C. albicans cell surface, particularly on the blastoconidia. Semiquantitative **flow cytometry** analysis showed the sensitivity of this GAPDH form to trypsin and its resistance to be removed with 2 M NaCl or 2% sodium dodecyl sulfate. The decrease in **fluorescence** in the presence of soluble GAPDH indicates the specificity of the labelling. In addition, a dose-dependent GAPDH enzymatic activity was detected in intact blastoconidia and germ tube cells. This activity was reduced by pretreatment of the cells with trypsin, formaldehyde, and PAb anti-CA-GAPDH. These observations indicate that an immunogenic, enzymatically active cell wall-associated form of the glycolytic enzyme GAPDH is found at the cell surface of C. albicans cells.

L5 ANSWER 2 OF 66 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 1998173052 MEDLINE
DOCUMENT NUMBER: 98173052 PubMed ID: 9514119
TITLE: Isolation of anti-T cell receptor scFv mutants by yeast surface display.
AUTHOR: Kieke M C; Cho B K; Boder E T; Kranz D M; Wittrup K D
CORPORATE SOURCE: Department of Biochemistry, University of Illinois, Urbana 61801, USA.
CONTRACT NUMBER: R01AI35990 (NIAID)
T32 GM07283 (NIGMS)
SOURCE: PROTEIN ENGINEERING, (1997 Nov) 10 (11) 1303-10. Journal code: PR1; 8801484. ISSN: 0269-2139.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199804
ENTRY DATE: Entered STN: 19980507
Last Updated on STN: 19980507
Entered Medline: 19980424

AB Yeast surface display and sorting by **flow cytometry** have been used to isolate mutants of an scFv that is specific for the Vbeta8 region of the T cell receptor. Selection was based on equilibrium binding by two fluorescently labeled probes, a soluble Vbeta8 domain and an antibody to the c-myc epitope tag present at the carboxy-terminus of the scFv. The mutants that were selected in this screen included a scFv with threefold increased affinity for the Vbeta8 and scFv clones that were bound with reduced affinities by the anti-c-myc antibody. The latter finding indicates that the yeast display system may be used to map conformational epitopes, which cannot be revealed by standard peptide screens. Equilibrium antigen binding constants were estimated within the surface display format, allowing screening of isolated mutants without necessitating subcloning and soluble expression. Only a relatively small **library** of yeast cells (3 x 10[5]) displaying randomly mutagenized scFv was screened to identify these mutants, indicating that this system will provide a powerful tool for engineering the binding properties of eucaryotic secreted and cell surface proteins.

L5 ANSWER 3 OF 66 MEDLINE
ACCESSION NUMBER: 97452346 MEDLINE
DOCUMENT NUMBER: 97452346 PubMed ID: 9308760
TITLE: Identification of an epitope of alpha-enolase (a candidate plasminogen receptor) by phage display.
AUTHOR: Arza B; Felez J; Lopez-Alemany R; Miles L A; Munoz-Canoves P
CORPORATE SOURCE: Institut de Recerca Oncologica, Hospital Duran i Reynals, Barcelona, Spain.
CONTRACT NUMBER: HL-38272 (NHLBI)
HL-45934 (NHLBI)
SOURCE: THROMBOSIS AND HAEMOSTASIS, (1997 Sep) 78 (3) 1097-103. Journal code: VQ7; 7608063. ISSN: 0340-6245.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199801

ENTRY DATE: Entered STN: 19980129
Last Updated on STN: 19980129
Entered Medline: 19980114

AB Alpha-enolase is an ubiquitous cytoplasmic glycolytic enzyme which also exhibits cell surface mediated functions and a structural role in the lens of some species. An alpha-enolase related molecule (alpha-ERM) is present on the surfaces of neutrophils, monocytes and monocytoid cells and has the capacity to specifically bind plasminogen, suggesting that alpha-ERM may function as a plasminogen receptor. We have generated a monoclonal antibody (mAB), 9C12, against alpha-ERM. This mAB reacted with both alpha-ERM and purified human alpha-enolase in Western blotting and in enzyme linked immunosorbent assays (ELISA). mAB 9C12 detected a cell surface associated molecule on human peripheral blood neutrophils and on U937 human monocytoid cells as assessed by **fluorescence** activated cell sorting (FACS) analyses. In addition, mAB 9C12 recognized an intracellular pool of alpha-enolase/alpha-ERM in permeabilized U937 cells. A phage display approach was employed to identify the alpha-enolase epitope recognized by mAB 9C12. Random fragments of 100-300 base pairs (bp), obtained from the full length human alpha-enolase cDNA, were cloned into the filamentous phage vector pComb3B, to generate a phage-displayed peptide **library**. Recombinant phages binding to mAB 9C12 were selected and their DNA inserts characterized by direct sequencing. All of the fragments which bound to mAB 9C12 encoded the common sequence DLDFKSPDDPSRYISP, spanning amino acids 257-272 of human alpha-enolase. This sequence is located within an external loop of the molecule. These data indicate that this sequence contains the epitope recognized by mAB 9C12 and is, therefore, exposed on the cell surface, further suggesting that alpha-enolase and alpha-ERM share common amino acid sequences.

L5 ANSWER 4 OF 66 MEDLINE
ACCESSION NUMBER: 97325526 MEDLINE
DOCUMENT NUMBER: 97325526 PubMed ID: 9181579
TITLE: Use of a modified bacteriophage to probe the interactions between peptides and ion channel receptors in mammalian cells.
AUTHOR: Li M
CORPORATE SOURCE: Department of Physiology, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA..
min-li@qmail.bs.jhu.edu
SOURCE: NATURE BIOTECHNOLOGY, (1997 Jun) 15 (6) 559-63.
Journal code: CQ3; 9604648. ISSN: 1087-0156.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199708
ENTRY DATE: Entered STN: 19970902
Last Updated on STN: 19970902
Entered Medline: 19970819

AB Besides natural peptide ligands, screening of random peptide libraries has yielded novel bioactive peptides for cell surface receptors. A method is described that uses a modified bacteriophage as a detection reagent to monitor the expression of receptor channels in mammalian cells and to probe the molecular interaction between phage-tethered peptides (phi T-peptides) and specific receptor targets. By taking advantage of a specific multivalent interaction between phi T-peptides and the receptor target, assays have been developed that use phi T-peptides specific for the N-methyl-D-aspartate glutamate receptor, an important ligand-gated ion channel in the nervous system, to monitor the receptor expression in cultured mammalian cells. Combining these phi T-peptide binding assays with **fluorescence**-activated cell sorting, 10(4) random glutamate receptor mutants were screened and candidate interaction residues were identified. This dual heterologous expression system offers a powerful approach to the molecular studies of protein-protein interactions.

L5 ANSWER 5 OF 66 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 97222477 MEDLINE
DOCUMENT NUMBER: 97222477 PubMed ID: 9069120
TITLE: Mapping of microsatellite markers developed from a flow-sorted swine chromosome 6 **library**.
AUTHOR: Grimm D R; Goldman T; Holley-Shanks R; Buoen L; Mendiola J; Schook L B; Louis C; Rohrer G A; Lunney J K
CORPORATE SOURCE: USDA-ARS-IDRL, BARC-East, Building 1040, Room 105, Beltsville, MD 20705, USA.
SOURCE: MAMMALIAN GENOME, (1997 Mar) 8 (3) 193-9.
Journal code: BES; 9100916. ISSN: 0938-8990.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-U24283; GENBANK-U24284; GENBANK-U24285; GENBANK-U24286; GENBANK-U62944; GENBANK-U62945; GENBANK-U62946; GENBANK-U62947; GENBANK-U62948; GENBANK-U62949; GENBANK-U62950; GENBANK-U62951; GENBANK-U62952; GENBANK-U62953; GENBANK-U62954; GENBANK-U62955; GENBANK-U62956; GENBANK-U62957; GENBANK-U62958; GENBANK-U62959; GENBANK-U66700
ENTRY MONTH: 199706
ENTRY DATE: Entered STN: 19970630
Last Updated on STN: 19970630
Entered Medline: 19970619

AB Swine Chromosome (Chr) 6-enriched libraries, generated with size-fractionated DNA isolated from chromosomes sorted by **flow cytometry**, have been used to develop new Chr 6 microsatellite markers. Chromosome isolation procedures were established to reproducibly prepare high quality chromosomes from phytohemagglutinin (PHA)-stimulated swine peripheral blood lymphocytes and to sort individual chromosomes after staining with Hoechst 33258 and chromomycin A3. Chromosome purity was verified by specific staining of swine Chr 6 with **fluorescence** in situ hybridization (FISH) by use of painting probes generated by degenerate oligonucleotide-primed polymerase chain reaction (DOP-PCR) amplification of as few as 300 sorted Chr 6. For **library** construction, DNA was extracted from flow-sorted pools representing Chr 6, amplified, size selected for fragments from 300 to 700 bp, and ligated

into pBluescript SK II+ or Lambda ZAP Express. The libraries were then screened with a radiolabeled poly-(dCA) DNA probe. Of 107 (CA)n repeat-containing clones verified by sequencing, 21 were polymorphic and used to genotype the University of Illinois swine reference families. Linkage analysis was then performed with CRIMAP 2.4 (LOD > 3.0), and the results showed that 15 of the microsatellites mapped to swine Chr 6. At least three of these new markers map to locations where there were gaps in the consensus Chr 6 map. Another four markers, because of their PIC values, should provide more informative markers in other areas of the map. Most of the new markers can also be used for automated genotyping with **fluorescent** labeling. This set of 15 new Chr 6 markers will, therefore, be useful in helping to define QTL associated with swine Chr 6.

L5 ANSWER 6 OF 66 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 97088951 EMBASE
DOCUMENT NUMBER: 1997088951
TITLE: Cell display **library** for gene cloning of variable regions of human antibodies to hepatitis B surface antigen.
AUTHOR: Higuchi K.; Araki T.; Matsuzaki O.; Sato A.; Kanno K.; Kitaguchi N.; Ito H.
CORPORATE SOURCE: K. Kanno, Pharmaceutical Group, Fundamental Res. Lab. of Life Sci., Asahi Chemical Industry Co., Ltd., 2-1 Samejima, Fuji, Shizuoka 416, Japan. a8012181@ut.asahi-kasei.co.jp
SOURCE: Journal of Immunological Methods, (1997) 202/2 (193-204). Refs: 27
ISSN: 0022-1759 CODEN: JIMMBG
PUBLISHER IDENT.: S 0022-1759(97)00010-0
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
022 Human Genetics
026 Immunology, Serology and Transplantation
LANGUAGE: English
SUMMARY LANGUAGE: English
AB A novel cell display system was developed for cloning the variable region (V) genes of antigen-specific human antibodies. The system is based on an antibody **library** displayed on the surface of COS cells, using a plasmid vector designed to direct expression of membrane-bound antibodies. COS cells expressing antigen-specific antibodies were separated using a flow cytometer for their binding to a **fluorescent** dye-labeled antigen. To test the performance of this system, we cloned V genes of 4 antibodies directed against hepatitis B surface antigen (HBsAg) from a **library** prepared from peripheral blood lymphocytes of a vaccinated donor. These membrane-bound anti-HBsAg antibodies were easily converted to soluble forms, all of which showed a size similar to human serum IgG in SDS-PAGE and the same specific binding to HBsAg as membrane-bound forms in ELISA. All V(H) and Y(.kappa.) gene segments of the 4 clones isolated in this study belonged to V(H)III and V(.kappa.)I subgroups, respectively. These findings demonstrate the potential and selection capabilities of our cell display system for cloning the V genes of antigen-specific human antibodies.

L5 ANSWER 7 OF 66 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 97362024 MEDLINE
DOCUMENT NUMBER: 97362024 PubMed ID: 9215560
TITLE: Chromosomal homeologies between human, harbor seal (Phoca vitulina) and the putative ancestral carnivore karyotype revealed by Zoo-FISH.
AUTHOR: Fronicke L; Muller-Navia J; Romanakis K; Scherthan H
CORPORATE SOURCE: Abteilung fur Humanbiologie und Humangenetik, Universitat Kaiserslautern, Postfach 3049, D-67653 Kaiserslautern, Germany.
SOURCE: CHROMOSOMA, (1997 Jul) 106 (2) 108-13.
Journal code: D7A; 2985138R. ISSN: 0009-5915.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199709
ENTRY DATE: Entered STN: 19970922
Last Updated on STN: 19970922
Entered Medline: 19970909
AB We report on the construction of the first comparative Zoo-FISH map of a marine mammal. Zoo-FISH with DNA probes from a human chromosome-specific **library** to metaphase spreads of the harbor seal (Phoca vitulina) disclosed 31 conserved syntenic segments covering the complete autosomal complement and the X chromosome. Comparison with Zoo-FISH maps of other species reveals that the harbor seal shares a high degree of karyotypic homeology with the human complement and an even higher degree with the conordinal cat complement. These findings suggest that pinniped, felid and human karyotypes have maintained conserved complements. Based on data of Zoo-FISH and comparative cytogenetics, a Zoo-FISH map of the ancestral carnivore karyotype (Z-CAR) is proposed. **Flow cytometry** revealed that the DNA value of the harbor seal genome is 79% that of the human genome.

L5 ANSWER 8 OF 66 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:333943 CAPLUS
DOCUMENT NUMBER: 127:3859
TITLE: An optimized method for cell-based phage display panning
AUTHOR(S): Watters, Jennifer M.; Telleman, Pieter; Junghans, Richard P.
CORPORATE SOURCE: Biotherapeutics Development Lab, Department of Medicine, Harvard Medical School, New England Deaconess Hospital, Boston, MA, 02215, USA
SOURCE: Immunotechnology (1997), 3(1), 21-29
CODEN: IOTEER; ISSN: 1380-2933
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Traditional methods of phage display panning, bind purified antigen to plates or other solid phases to which libraries are then applied, followed by vigorous washings in detergent-supplemented buffers to select for

specific phage Fab. These methods are not directly applicable to antigens in their native environment on cell surfaces or in settings where the target antigen is unknown. To develop a model antigen system employing whole cells rather than purified protein immobilized on a substrate; to optimize methods for phage display panning using a cell-based system. Specificity of binding of phage Fab to antigen on cells was demonstrated by output titer and by **flow cytometry**. Output titers showed a plateau and binding advantage, after four washes, corresponding to removal of most non-specifically bound phage. Enrichment advantage was independent of input phage no. Longer incubation times, to cell tolerance, improved specific binding. Temp. had modest impact as a variable in the panning and washing. An increase in output titers paralleled enrichment for specific phage Fab. An optimized method applied to whole cells can productively enrich specific phage Fab in mixts. with large excesses of non-specific phage Fab over several rounds of panning.

L5 ANSWER 9 OF 66 MEDLINE
ACCESSION NUMBER: 96279212 MEDLINE
DOCUMENT NUMBER: 96279212 PubMed ID: 8663178
TITLE: Rapid identification of phosphopeptide ligands for SH2 domains. Screening of peptide libraries by **fluorescence**-activated bead sorting.
AUTHOR: Muller K; Gombert F O; Manning U; Grossmuller F; Graff P; Zaegel H; Zuber J F; Freuler F; Tschopp C; Baumann G
CORPORATE SOURCE: Sandoz Pharma Ltd., Preclinical Research, CH-4002 Basel, Switzerland.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1996 Jul 12) 271 (28) 16500-5.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199608
ENTRY DATE: Entered STN: 19960911
Last Updated on STN: 19960911
Entered Medline: 19960829

AB A method for the identification of high-affinity ligands to SH2 domains by **fluorescence**-activated bead sorting (FABS) was established. Recombinant SH2 domains, expressed as glutathione S-transferase (GST) fusion proteins, were incubated with a phosphotyrosine (Y*)-containing peptide **library**. 6.4 x 10(5) individual peptides of nine amino acids in length (EPX6Y*X19X7X19X7X6) were each displayed on beads. Phosphopeptide interaction of a given SH2 domain was monitored by binding of fluorescein isothiocyanate-labeled antibodies directed against GST. High-**fluorescence** beads were isolated by flow cytometric sorting. Subsequent pool sequencing of the selected beads revealed a distinct pattern of phosphotyrosine-containing motifs for each individual SH2 domain: the SH2 domain of the adapter protein Grb2 predominantly selected beads with the sequence Y*ENDP, whereas the C-terminal SH2 domain of the tyrosine kinase Syk selected Y*EELD, each motif representing the most frequently found residues C-terminal to the phosphotyrosine. For deconvolution studies, soluble phosphopeptides comprising variations of the Grb2 motifs were resynthesized and analyzed by surface plasmon resonance.

L5 ANSWER 10 OF 66 MEDLINE
ACCESSION NUMBER: 96234073 MEDLINE
DOCUMENT NUMBER: 96234073 PubMed ID: 8650202
TITLE: Molecular cloning of the Golgi apparatus uridine diphosphate-N-acetylglucosamine transporter from Kluyveromyces lactis.
AUTHOR: Abeijon C; Robbins P W; Hirschberg C B
CORPORATE SOURCE: Department of Biochemistry and Molecular Biology, University of Massachusetts Medical Center, Worcester, 01655, USA.
CONTRACT NUMBER: GM 30365 (NIGMS)
GM 45188 (NIGMS)
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1996 Jun 11) 93 (12) 5963-8.
Journal code: PV3; 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-U48413
ENTRY MONTH: 199607
ENTRY DATE: Entered STN: 19960805
Last Updated on STN: 19960805
Entered Medline: 19960725

AB The mannan chains of Kluyveromyces lactis mannoproteins are similar to those of Saccharomyces cerevisiae except that they lack mannose phosphate and have terminal alpha1-->2-linked N-acetylglucosamine. The biosynthesis of these chains probably occurs in the lumen of the Golgi apparatus, by analogy to S. cerevisiae. The sugar donors, GDP-mannose and UDP-GlcNAc, must first be transported from the cytosol, their site of synthesis, via specific Golgi membrane transporters into the lumen where they are substrates in the biosynthesis of these mannoproteins. A mutant of K. lactis, mnn2-2, that lacks terminal N-acetylglucosamine in its mannan chains in vivo, has recently been characterized and shown to have a specific defect in transport of UDP-GlcNAc into the lumen of Golgi vesicles in vitro. We have now cloned the gene encoding the K. lactis Golgi membrane UDP-GlcNAc transporter by complementation of the mnn2-2 mutation. The mnn2-2 mutant was transformed with a genomic **library** from wild-type K. lactis in a pKD1-derived vector; transformants were isolated and phenotypic correction was monitored following cell surface labeling with fluorescein isothiocyanate conjugated to Griffonia simplicifolia II lectin, which binds terminal N-acetylglucosamine, and a **fluorescent** activated cell sorter. A 2.4-kb DNA fragment was found to restore the wild-type lectin binding phenotype. Upon loss of the plasmid containing this fragment, reversion to the mutant phenotype occurred. The above fragment contained an open reading frame for a multitransmembrane spanning protein of 328 amino acids. The protein contains a leucine zipper motif and has high homology to predicted

proteins from S. cerevisiae and C. elegans. In an assay in vitro, Golgi vesicles isolated from the transformant had regained their ability to transport UDP-GlcNAc. Taken together, the above results strongly suggest that the cloned gene encodes the Golgi UDP-GlcNAc transporter of K. lactis.

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YOU HAVE REQUESTED DATA FROM 56 ANSWERS - CONTINUE? Y/(N):y

L5 ANSWER 11 OF 66 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 96343366 EMBASE
DOCUMENT NUMBER: 1996343366
TITLE: Large DNA fragment sizing by **flow cytometry**: Application to the characterization of P1 artificial chromosome (PAC) clones.
AUTHOR: Huang Z.; Petty J.T.; O'Quinn B.; Longmire J.L.; Brown N.C.; Jett J.H.; Keller R.A.
CORPORATE SOURCE: Chemical Science Technology Division, Los Alamos National Laboratory,Los Alamos, NM 87545, United States
SOURCE: Nucleic Acids Research, (1996) 24/21 (4202-4209). ISSN: 0305-1048 CODEN: NARHAD
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB A **flow cytometry**-based, ultrasensitive **fluorescence** detection technique is used to size individual DNA fragments up to 167 kb in length. Application of this technology to the sizing of P1 artificial chromosomes (PACs) in both linear and supercoiled forms is described. It is demonstrated that this method is well suited to characterizing PAC/BAC clones and will be very useful for the analysis of large insert libraries. **Fluorescence** bursts are recorded as individual, dye stained DNA fragments pass through a low power, focused, continuous laser beam. The magnitudes of the **fluorescence** bursts are linearly proportional to the lengths of the DNA fragments. The histograms of the burst sizes are generated in < 3 min with < 1 pg of DNA. Results on linear fragments are consistent with those obtained by pulsed-field gel electrophoresis. In comparison with pulsed-field gel electrophoresis, sizing of large DNA fragments by this approach is more accurate, much faster, requires much less DNA, and is independent of the DNA conformation.

L5 ANSWER 12 OF 66 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 97084577 MEDLINE
DOCUMENT NUMBER: 97084577 PubMed ID: 8930920
TITLE: Bacterial genetics by **flow cytometry**: rapid isolation of Salmonella typhimurium acid-inducible promoters by differential **fluorescence** induction.
AUTHOR: Valdivia R H; Falkow S
CORPORATE SOURCE: Department of Microbiology and Immunology, Stanford University School of Medicine, California 94305, USA.. valdivia@cmgm.stanford.edu
CONTRACT NUMBER: AI 26195 (NIAID)
DK 38707 (NIDDK)
SOURCE: MOLECULAR MICROBIOLOGY, (1996 Oct) 22 (2) 367-78. Journal code: MOM; 8712028. ISSN: 0950-382X.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-U62708; GENBANK-U62709; GENBANK-U62710; GENBANK-U62711; GENBANK-U62712; GENBANK-U62713; GENBANK-U62714
ENTRY MONTH: 199702
ENTRY DATE: Entered STN: 19970306
Last Updated on STN: 19980206
Entered Medline: 19970225
AB The ability of Salmonella typhimurium to survive and replicate within murine macrophages is dependent on a low phagosomal pH. This requirement for an acidic vacuole suggests that low pH is an important environmental stimulus for the transcription of genes necessary for intracellular survival. To study the behaviour of acid-inducible genes in response to the phagosomal environment, we have applied a novel enrichment strategy, termed differential **fluorescence** induction (DFI), to screen an S. typhimurium **library** for promoters that are upregulated at pH 4.5. DFI utilizes a **fluorescence**-enhanced green **fluorescent** protein (GFP) and a **fluorescence**-activated cell sorter (FACS) to perform genetic selection. In the presence of an inducing stimulus, such as low pH, a FACS is used to sort highly **fluorescent** bacterial clones bearing random promoters fused to the mutant GFP protein (GFPmut). This population is then amplified at neutral pH and the least **fluorescent** population is sorted. Sequential

sorts for **fluorescent** and non-**fluorescent** bacteria in the presence or absence of inducing conditions rapidly enriches for promoter fusions that are regulated by the inducing stimulus. We have identified eight acid-inducible promoters and quantified their expression in response to pH 4.5 and to the phagosome milieu. These acid-inducible promoters exhibited extensive homology to promoter regions of genes encoding for cell-surface-maintenance enzymes, stress proteins, and generalized efflux pumps. Only a subset of these promoters was induced in macrophages with kinetics and levels of expression that do not necessarily correlate with in vitro pH-shock induction. This suggests that while low pH is a relevant inducer of intracellular gene expression, additional stimuli in the macrophage can modulate the expression of acid-inducible genes.

L5 ANSWER 13 OF 66 MEDLINE DUPLICATE 6
ACCESSION NUMBER: 97129113 MEDLINE
DOCUMENT NUMBER: 97129113 PubMed ID: 8973653
TITLE: Isolation and characterization of a 14.5-kDa trichloroacetic-acid-soluble translational inhibitor protein from human monocytes that is upregulated upon cellular differentiation.
AUTHOR: Schmiedeknecht G; Kerkhoff C; Orso E; Stohr J; Aslanidis C; Nagy G M; Knuechel R; Schmitz G
CORPORATE SOURCE: Institute of Clinical Chemistry and Laboratory Medicine, University of Regensburg, Germany.
SOURCE: EUROPEAN JOURNAL OF BIOCHEMISTRY, (1996 Dec 1) 242 (2) 339-51.
Journal code: EMZ; 0107600. ISSN: 0014-2956.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-X95384
ENTRY MONTH: 199701
ENTRY DATE: Entered STN: 19970219
Last Updated on STN: 19970219
Entered Medline: 19970128

AB A trichloroacetic-acid-soluble 14.5-kDa protein (p14.5) has been isolated from human mononuclear phagocytes (MNP) by a combination of trichloroacetic acid extraction, preparative electrophoresis and hydrophobic affinity chromatography; five tryptic peptides were subjected to protein sequencing. The full-length cDNA of the protein was cloned and sequenced from a lambda gt11 human liver **library**. The cDNA showed a remarkable similarity to a rat protein preferentially expressed in hepatocytes and renal tubular epithelial cells. The encoded protein is 137 amino acids long and similar to members of a new hypothetical family of small proteins with presently unknown function, named YER057c/YJGF. Human recombinant p14.5 inhibits in vitro protein synthesis in a rabbit reticulocyte lysate system. Unlike other inhibitors of protein synthesis, p14.5 is not phosphorylated despite the presence of putative phosphorylation sites. The p14.5 mRNA is weakly expressed in freshly isolated monocytes but is significantly upregulated when these monocytes are subjected to differentiation. This is also reflected by a differentiation-dependent increase in the protein concentration as demonstrated by immunoblots from cytosolic fractions and **fluorescence**-activated **flow cytometry** of permeabilized cells. A differentiation-dependent mRNA and protein expression of p14.5 is further suggested by the observation of a low expression in a variety of liver and kidney tumor cells and a high expression in fully differentiated cells as assessed by immunohistochemistry and northern blots. The highest mRNA expression was found in hepatocytes and renal distal tubular epithelial cells and only weak expression was found in other human tissues as evaluated by northern blot analysis. The preferential localization of the immunoreaction product seemed to be cytoplasmatic but, in less differentiated cells, nuclear labeling was occasionally visible. Immunoblotting of subcellular fractions confirmed these data. The high degree of evolutionary conservation of p14.5, the considerable upregulation during cellular differentiation and its potential role as a translational inhibitor may reflect an involvement in basic cellular mechanisms, e.g. a differentiation-dependent regulation of protein synthesis in hepatocytes, renal tubular epithelial cells, smooth muscle cells and MNP.

L5 ANSWER 14 OF 66 MEDLINE DUPLICATE 7
ACCESSION NUMBER: 96200387 MEDLINE
DOCUMENT NUMBER: 96200387 PubMed ID: 8927696
TITLE: Cytogenetic damage and the radiation-induced G1-phase checkpoint.
AUTHOR: Gupta N; Vij R; Haas-Kogan D A; Israel M A; Deen D F; Morgan W F
CORPORATE SOURCE: Brain Tumor Research Center of the Department of Neurological Surgery, University of California San Francisco 94143, USA.
CONTRACT NUMBER: CA13525 (NCI)
SOURCE: RADIATION RESEARCH, (1996 Mar) 145 (3) 289-98.
Journal code: QMP; 0401245. ISSN: 0033-7587.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199611
ENTRY DATE: Entered STN: 19961219
Last Updated on STN: 19961219
Entered Medline: 19961114

AB It is proposed that genomic integrity is preserved after DNA damage in a variety of ways. X irradiation induces a p53-dependent G1-phase cell cycle checkpoint which putatively allows time for repair of DNA damage. The p53 protein is also involved in the initiation of apoptosis after radiation-induced DNA damage, presumably leading to the elimination of lethally damaged cells from the irradiated population. To test the hypothesis that repair occurs in the additional time provided by the activation of the G1-phase checkpoint, we investigated whether the presence of a G1-phase arrest modified the frequency and type of chromosomal rearrangements at the first mitosis after irradiation.

Isogenic cell lines derived from the same human glioma cell line, but differing in p53 status, were used. Purified G1-phase cells, isolated by centrifugal elutriation and X-irradiated, were studied. The wild-type p53 cell line demonstrated a dose-dependent arrest during G1 phase, as determined by **flow cytometry**. These cells remained in G1-phase as long as 48 h after irradiation. Cells expressing a dominant-negative p53 mutation accumulated to a much lesser extent in G1 phase after irradiation. Cells lacking the G1-phase checkpoint showed increased survival at all radiation doses. There were no significant differences in the type or frequency of total chromosomal aberrations in mitotic cells from either cell line after 1,2,4 or 6 Gy X rays, as measured by conventional cytogenetic analysis. There was an increase, however, in the number of reciprocal translocations in mitotic cells with mutant p53 (lacking a G1-phase checkpoint), as measured by **fluorescence** in situ hybridization with a chromosome 4-specific DNA **library**, but only after 6 Gy. The results suggest that the presence of a well-defined p53-dependent G1-phase arrest does not reduce chromosomal aberrations caused by low doses of ionizing radiation markedly, but may reduce the overall degree of survival by triggering other G1-phase events.

L5 ANSWER 15 OF 66 MEDLINE
ACCESSION NUMBER: 97130619 MEDLINE
DOCUMENT NUMBER: 97130619 PubMed ID: 8976382
TITLE: Characterization of a flow-sorted human chromosome 10 cosmid **library** by FISH.
AUTHOR: Ma N S; Zheng C; Benchekroun Y; Deaven L L; Longmire J L; Moir D T; Mao J
CORPORATE SOURCE: Department of Human and Molecular Genetics, Genome Therapeutics Corporation, Waltham, USA.
CONTRACT NUMBER: R01-HG00122 (NHGRI)
R44-HG00491 (NHGRI)
SOURCE: CYTOGENETICS AND CELL GENETICS, (1996) 74 (4) 266-71.
Journal code: DXK; 0367735. ISSN: 0301-0171.
PUB. COUNTRY: Switzerland
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GDB-G00631948
ENTRY MONTH: 199701
ENTRY DATE: Entered STN: 19970128
Last Updated on STN: 19980206
Entered Medline: 19970115

AB Fluoresence in situ hybridization (FISH) was used to localize cosmids to regions of human chromosome 10. A total of 301 cosmids were selected randomly from a flow-sorted human chromosome 10 cosmid **library** constructed from human x hamster cell line 762-8A and arrayed in microtiter storage dishes. Over 70% (211/301) of the cosmids mapped to unique regions of chromosome 10. About 7% (22/301) produced multiple hybridization signals indicative of chimeric clones or sequences repeated at low copy number. Three cosmids (3/301, or 1%) hybridized to the centromeric regions of chromosome 10 and one or more other human chromosomes. About 19% (59/301) consisted mostly or entirely of hamster DNA inserts, and about 2% (6/301) appeared to be nonrecombinants.

L5 ANSWER 16 OF 66 MEDLINE
ACCESSION NUMBER: 1998040649 MEDLINE
DOCUMENT NUMBER: 98040649 PubMed ID: 9373318
TITLE: Separation of E. coli expressing functional cell-wall bound antibody fragments by FACS.
AUTHOR: Fuchs P; Weichel W; Dubel S; Breitling F; Little M
CORPORATE SOURCE: Recombinant Antibody Research Group, Deutsches Krebsforschungszentrum, Heidelberg, Germany.
SOURCE: IMMUNOTECHNOLOGY, (1996 Jun) 2 (2) 97-102.
Journal code: CR0; 9511979. ISSN: 1380-2933.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199712
ENTRY DATE: Entered STN: 19980109
Last Updated on STN: 19980109
Entered Medline: 19971210

AB BACKGROUND: The rapid development of recombinant antibody technology in the last few years has facilitated the generation of antibody libraries in bacteria. Recombinant antibodies against various antigens have been selected from these libraries by presenting each antibody on the surface of a phagemid particle that contains the antibody's gene. An alternative screening system is the display of antibody fragments on bacteria. A major advantage is the possibility to select single cells directly from a large number of bacteria by using fluorescently labeled antigens and **fluorescence** assisted cell sorting (FACS). OBJECTIVES: pAP is an expression vector for the bacterial display of antibody fragments. E. coli transformed with pAP express a single chain antibody (scFv) fused to the peptidoglycan-associated-lipoprotein (PAL). This fusion protein binds strongly to the cell wall. To employ this system for screening, we have investigated the possibility of selecting antigen-specific clones by FACS. STUDY DESIGN AND RESULTS: Several DNA fragments coding for various scFvs were inserted into the pAP expression vector. E. coli were transformed with these plasmids and immunostained with **fluorescent** antigens under given conditions. We were able to select stained E. coli expressing a specific scFv from unstained E. coli expressing a non-binding scFv by FACS. The specific selection of the bacteria was demonstrated by amplifying their genes by PCR. CONCLUSIONS: Conditions are described for separating E. coli containing scFv bound to their cell wall by FACS using fluorescently labeled antigens. These studies provide a basis for screening libraries of scFv antibodies.

L5 ANSWER 17 OF 66 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1996:217232 CAPLUS
TITLE: Flow cytometric screening of antibody fragment contact residue libraries displayed at the surface of escherichia coli: A model system for the development of bacterial surface display for antibody selection.

AUTHOR(S): Daugherty, Patrick; Chen, Gang; Iverson, Brent; Georgiou, George
CORPORATE SOURCE: Departments Chemical Engineering, University Texas, Austin, TX, 78712, USA
SOURCE: Book of Abstracts, 211th ACS National Meeting, New Orleans, LA, March 24-28 (1996), BIOT-076. American Chemical Society: Washington, D. C.
CODEN: 62PIAJ
DOCUMENT TYPE: Conference; Meeting Abstract
LANGUAGE: English

AB The design of antibodies and peptides for particular applications has been made possible in recent years through the advent of various **library** generation and screening technologies. In particular, the display of libraries of antibody fragments on the external surface of filamentous phage as fusions to major coat proteins has yielded antibody fragments and peptides for numerous applications. However, the screening of antibody libraries by phage display has proven to be technol. challenging and suffers from certain limitations. Recently, we have shown that single-chain antibodies (scFv) may be displayed at the surface of Escherichia coli, and subsequently that clones as rare as one in 105 may be selected from cells displaying non-antigen binding proteins. In order to demonstrate the potential of bacterial surface display in conjunction with **fluorescence** activated cell sorting (FACS), for high-affinity antibody fragment selection, we have constructed small antibody libraries displayed at the surface of Escherichia coli, and screened them for high-affinity antigen binding. Several plasmid libraries encoding surface-displayed single-chain antibody fragments have been generated by PCR randomization of key antigen-contact residues in the digoxin scFv, as suggested by the known crystal structure. The plasmid libraries were transformed into Escherichia coli and screened for high affinity antigen binding by incubation of **library** aliquots with a **fluorescent** antigen conjugate followed by FACS. Amplification of the sorted populations yielded populations enriched for high affinity antigen binding, as detd. by **flow cytometry** and DNA sequence anal. of individual clones. Several important parameters for bacterial surface display, **library** construction, and FACS screening have been analyzed and will be presented.

L5 ANSWER 18 OF 66 MEDLINE
ACCESSION NUMBER: 97150954 MEDLINE
DOCUMENT NUMBER: 97150954 PubMed ID: 8995490
TITLE: Development of DNA libraries specific for Chinese hamster chromosomes 3, 4, 9, 10, X, and Y by DOP-PCR.
AUTHOR: Xiao Y; Slijepcevic P; Arkesteijn G; Darroudi F; Natarajan A T
CORPORATE SOURCE: MGC-Department of Radiation Genetics and Chemical Mutagenesis, Leiden University, The Netherlands.
SOURCE: CYTOGENETICS AND CELL GENETICS, (1996) 75 (1) 57-62. Journal code: DXK; 0367735. ISSN: 0301-0171.
PUB. COUNTRY: Switzerland
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199701
ENTRY DATE: Entered STN: 19970219
Last Updated on STN: 19970219
Entered Medline: 19970130

AB In a previous study of ours, DNA libraries specific for Chinese hamster chromosomes 1, 2, 3, 5, 6, 7, and 8 were constructed by single laser flow sorting and the linker adapter PCR method. Since a single laser flow sorting of chromosomes is based on chromosome size, it is difficult to separate chromosomes of equal size, such as chromosome 3 and the X chromosome. This problem can be circumvented by bivariate flow sorting. In the present study, we have used bivariate flow sorting of Chinese hamster chromosomes and the degenerate oligonucleotide-primed polymerase chain reaction (DOP-PCR) for generating DNA libraries specifically for chromosomes 3, 4, 9, 10, X, and Y. **Fluorescence** in situ hybridization (FISH) analyses indicate that the probe libraries are highly specific and can be applied to various cytogenetic studies.

L5 ANSWER 19 OF 66 MEDLINE
ACCESSION NUMBER: 96305137 MEDLINE
DOCUMENT NUMBER: 96305137 PubMed ID: 8707053
TITLE: FACS-optimized mutants of the green **fluorescent** protein (GFP).
AUTHOR: Cormack B P; Valdivia R H; Falkow S
CORPORATE SOURCE: Department of Microbiology and Immunology, Stanford University School of Medicine, CA 94305-5402, USA.. cormack@cmgm.stanford.edu
CONTRACT NUMBER: AI 36396 (NIAID)
SOURCE: GENE, (1996) 173 (1 Spec No) 33-8. Journal code: FOP; 7706761. ISSN: 0378-1119.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-U73901; GENBANK-U89685; GENBANK-U89686
ENTRY MONTH: 199609
ENTRY DATE: Entered STN: 19960919
Last Updated on STN: 19980206
Entered Medline: 19960911

AB We have constructed a **library** in Escherichia coli of mutant gfp genes (encoding green **fluorescent** protein, GFP) expressed from a tightly regulated inducible promoter. We introduced random amino acid (aa) substitutions in the twenty aa flanking the chromophore Ser-Tyr-Gly sequence at aa 65-67. We then used **fluorescence**-activated cell sorting (FACS) to select variants of GFP that fluoresce between 20-and 35-fold more intensely than wild type (wt), when excited at 488 nm. Sequence analysis reveals three classes of aa substitutions in GFP. All three classes of mutant proteins have highly shifted excitation maxima. In addition, when produced in E. coli, the folding of the mutant proteins is more efficient than folding of wt GFP. These two properties contribute to a greatly increased (100-fold) **fluorescence** intensity, making the mutants useful for a number of applications.

L5 ANSWER 20 OF 66 MEDLINE
ACCESSION NUMBER: 97381297 MEDLINE
DOCUMENT NUMBER: 97381297 PubMed ID: 9238629
TITLE: The use of a combinatorial **library** method to isolate human tumor cell adhesion peptides.
AUTHOR: Pennington M E; Lam K S; Cress A E
CORPORATE SOURCE: Department of Radiation Oncology, University of Arizona, Tucson 85824, USA.
CONTRACT NUMBER: CA 17094 (NCI)
CA 23047 (NCI)
CA 56666 (NCI)
SOURCE: MOLECULAR DIVERSITY, (1996 Oct) 2 (1-2) 19-28.
Journal code: CWL; 9516534. ISSN: 1381-1991.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199708
ENTRY DATE: Entered STN: 19970902
Last Updated on STN: 19970902
Entered Medline: 19970821

AB Tumor cell progression is dependent in part on the successful adhesive interactions of the cells with the extracellular matrix. In this study, a new approach is described to isolate linear peptide ligand candidates involved in cellular adhesion. A synthetic combinatorial peptide **library** based on the 'one-bead-one-peptide' concept was incubated with live human prostate cancer cells for 90 min at 37 degrees C. The peptide bead coated with a monolayer of cells was then isolated for microsequencing. The DU145 (DU-H) cells were chosen since they have been previously characterized as containing elevated levels of a laminin receptor for cell adhesion, the alpha 6 beta 1 integrin on the cell surface. The use of a function-blocking antibody (GoH3) allows for the detection of peptides which are alpha 6-specific ligand candidates. From two different libraries (linear 9-mer and 11-mer) of a total of 1,500,000 beads, 68 peptide beads containing attached cells were isolated. These positive beads were then retested to determine the ability of the GoH3 antibody to block binding of the cells to the peptide beads. The alpha 6 integrin candidate peptide beads (five in total) were recovered and two of the beads were microsequenced. These two peptides, RU-1 (LNIVS-VNGRHX) and RX-1 (DNRIRLQAKXX), resemble the previously reported active peptide sequences (GD-2 and AG-73) from native laminin. The RU-1, RX-1 and AG-73 peptides were tested for their ability to support cell attachment and to bind the cell surface of DU-H prostate carcinoma cells in suspension using **fluorescence**-activated cell-sorting (FACS) analysis. Both RU-1 and AG-73 peptides supported cellular attachment within 1 h. In contrast, after 1 h, EHS laminin supported both cellular attachment and spreading. The RX-1 peptide exhibited only weak binding to the DU-H prostate carcinoma cells. FACS analysis indicated that AG-73 peptide attached to tumor cell surfaces over a range of concentrations, whereas the RU-1 peptide showed a homogeneous concentration required for attachment. The described strategy for screening a random peptide **library** offers three advantages: (i) ligands for conformationally sensitive receptors of adhesion can be isolated using live cells; (ii) specific binding can be selected for using function-blocking antibodies; and (iii) peptides supporting adhesion independent of spreading properties can be distinguished. In principle, specific adhesive peptides without prior knowledge of the sequence could be isolated for any epithelial cell surface receptor for which a function-blocking reagent is available.

L5 ANSWER 21 OF 66 MEDLINE
ACCESSION NUMBER: 95399346 MEDLINE
DOCUMENT NUMBER: 95399346 PubMed ID: 7669720
TITLE: Positive selection of growth-inhibitory genes.
AUTHOR: Maines J Z; Sunnarborg A; Rogers L M; Mandavilli A; Spielmann R; Boyd F T
CORPORATE SOURCE: Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis 55455, USA.
CONTRACT NUMBER: CA-56967 (NCI)
SOURCE: CELL GROWTH AND DIFFERENTIATION, (1995 Jun) 6 (6) 665-71.
Journal code: AYH; 9100024. ISSN: 1044-9523.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199510
ENTRY DATE: Entered STN: 19951026
Last Updated on STN: 19951026
Entered Medline: 19951019

AB We have isolated a limited set of cDNAs that limit cell proliferation using a unique assay based on the dilution of a lipophilic **fluorescent** dye as transfected cells divide. The identification of growth-inhibitory factors has been limited by the lack of a strong assay for growth inhibitors. A growth-inhibited cell does not grow and so is at a selective disadvantage in vitro when compared with any growing cell. Several assays have been used to screen for growth-inhibitory genes; however, these approaches are either very difficult to implement, leaky, or not comprehensive. We have developed an assay that selects for cDNAs capable of inhibiting proliferation in which cells are nonspecifically labeled with a lipophilic **fluorescent** dye, PKH-2, and subsequently transfected with a cDNA **library** made from growth-inhibited cells. With each cell division, the amount of dye per cell is reduced by one-half. Over time, growth-inhibited cells will retain more dye per cell relative to actively growing cells. The population is then analyzed by **fluorescence**-activated cell sorting, and the brightest cells in the population are isolated. This assay has allowed us to select pools of cDNAs enriched for growth-inhibitory activities and may provide a general method for identifying growth-inhibitory genes active in varying biological contexts. We report here the successful application of the dye retention assay to the selection of cDNAs that inhibit epithelial cell proliferation.

L5 ANSWER 22 OF 66 MEDLINE
ACCESSION NUMBER: 95248551 MEDLINE

DOCUMENT NUMBER: 95248551 PubMed ID: 7731047
TITLE: Selection and application of human single chain Fv antibody fragments from a semi-synthetic phage antibody display **library** with designed CDR3 regions.
AUTHOR: de Kruif J; Boel E; Logtenberg T
CORPORATE SOURCE: Department of Immunology, University of Utrecht, The Netherlands.
SOURCE: JOURNAL OF MOLECULAR BIOLOGY, (1995 Apr 21) 248 (1) 97-105.
Journal code: J6V; 2985088R. ISSN: 0022-2836.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-X83616; GENBANK-X83712; GENBANK-X83713; GENBANK-X83714
ENTRY MONTH: 199505
ENTRY DATE: Entered STN: 19950608
Last Updated on STN: 19960129
Entered Medline: 19950526

AB We have constructed a large (3.6 x 10(8) clones) phage display **library** of human single chain Fv (scFv) antibody fragments by combining 49 germline VH genes with synthetic heavy chain CDR3 (HCDR3) regions and seven light chains. The HCDR3 regions varied in length between 6 and 15 residues and were designed to contain fully randomized stretches of amino acid residues flanked by regions of limited residue variability that were composed of amino acid residues that frequently occur in natural antibodies. We reasoned that this approach would increase the frequency of functional molecules in our **library** and, in addition, permit us to efficiently utilize available cloning space. By direct selection on solid phase-bound antigens we obtained phage antibodies with binding activities to 13 different antigens, including Von Willebrand factor, the DNA-binding HMG box of transcription factor TCF-1 and the tumor antigen EGP-2. In addition, we applied a competitive selection procedure to target phage antibodies to the desired portion of a recombinant fusion protein and to select phage antibodies capable of discriminating between the two highly homologous homeobox proteins PBX1a and PBX2. The functional capacity of monoclonal phage antibodies was assessed in immuno-histochemical staining of tissue specimens. Western blotting assays and immunofluorescent analysis of cells by **flow cytometry**. The results demonstrate that this large human phage antibody **library** contains a broad assortment of binding specificities that can be applied in a variety of biochemical assays.

L5 ANSWER 23 OF 66 MEDLINE
ACCESSION NUMBER: 96159517 MEDLINE
DOCUMENT NUMBER: 96159517 PubMed ID: 8574897
TITLE: Characterization of a human chromosome 22 enriched bacterial artificial chromosome sublibrary.
AUTHOR: Kim U J; Shizuya H; Chen X N; Deaven L; Speicher S; Solomon J; Korenberg J; Simon M I
CORPORATE SOURCE: Division of Biology and Beckmann Institute, California Institute of Technology, Pasadena 91125, USA.
SOURCE: GENETIC ANALYSIS, (1995 Oct) 12 (2) 73-9.
Journal code: CEK; 9509403.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199603
ENTRY DATE: Entered STN: 19960321
Last Updated on STN: 19960321
Entered Medline: 19960312

AB Selection of chromosomal sublibraries from total human genomic libraries is critical for chromosome-based physical mapping approaches. We have previously reported a method of screening total human genomic **library** using flow sorted chromosomal DNA as a hybridization probe and selection of a human chromosome 22-enriched sublibrary from a total human bacterial artificial chromosome (BAC) **library** (Nucleic Acids Res 1995; 23: 1838-39). We describe here further details of the method of construction as well as characterization of the chromosome 22-enriched sublibrary thus constructed. Nearly 40% of the BAC clones that have been mapped by **fluorescence** in situ hybridization (FISH) analysis were localized to chromosome 22. By screening the sublibrary using chromosome 22-specific hybridization probes, we estimated that the sublibrary represents at least 2.5 x coverage of chromosome 22. This is in good agreement with the results from FISH mapping experiments. FISH map data also indicate that chromosome 22-specific BACs in the sublibrary represent all the subregions of chromosome 22.

L5 ANSWER 24 OF 66 AGRICOLA
ACCESSION NUMBER: 95:52968 AGRICOLA
DOCUMENT NUMBER: CAT10701060
TITLE: Immunocytochemical methods and protocols.
AUTHOR(S): Javois, Lorette C.
AVAILABILITY: DNAL (QH506.M45 no.34)
LC CONTROL NO.: 94-22475
SOURCE: c1994 xii, 435 p. : ill. ; 24 cm
Publisher: Totowa, N.J. : Humana Press, c1994.
Series: Methods in molecular biology (Clifton, N.J.) ; 34.
ISBN: 089603285X (acid-free paper).
NOTE: Includes bibliographical referemces and index.
Antibody preparation -- Overview of antibody use in immunocytochemistry -- Purification of antibodies using ammonium sulfate fractionation or gel filtration -- Purification of antibodies using ion-exchange chromatography -- Purification of antibodies using affinity chromatography -- Purification of antibodies using protein A-sepharose and FPLC -- Conjugation of fluorochromes to antibodies -- Biotinylation of antibodies -- Tissue preparation for light microscopic analyses -- Overview of cell fixation and permeabilization.
Preparation of frozen sections for analysis --

Processing of cell-touch preparations -- Processing of cytological specimens -- Processing of tissue-culture cells -- Processing of tissue specimens -- Light microscopic detection systems -- Overview of fluorophores -- Direct immunofluorescent labeling of cells -- **Fluorescence** labeling of surface antigens of attached or suspended tissue-culture cells -- **Fluorescence** labeling of intracellular antigens of attached or suspended tissue-culture cells.
Fluorescent labeling of surface or intracellular antigens in whole-amounts -- Overview of antigen detection through enzymatic activity -- Peroxidase-antiperoxidase (PAP) method -- Avidin-biotin complex (ABC) method -- Labeled avidin binding (LAB) method -- Avidin-biotin labeling of cellular antigens in cryostat-sectioned tissue -- ELISA on attachment-dependent or suspension grown cells -- Use of immunogold with silver enhancement -- **Fluorescence**-activated cell sorter (FACS) analyses -- Overview of **flow cytometry** and **fluorescent** probes for cytometry.
Tissue disaggregation -- Indirect immunofluorescent labeling of viable cells -- Indirect immunofluorescent labeling of fixed cells -- **Fluorescent** labeling of DNA -- Deparaffinization and processing of pathologic material -- Assay for phagocytosis -- Assay for filamentous actin -- Assay for chemoattractant binding -- Assay for oxidative metabolism -- Colloidal gold detection systems for electron microscopic analyses -- Fixation and embedding -- Preparation of colloidal gold -- Conjugation of colloidal gold to proteins.
Colloidal gold/streptavidin methods -- Pre-embedding labeling methods -- Postembedding labeling methods -- Photography -- Overview of **fluorescence** photomicrography -- Special applications -- Overview of **fluorescence** analysis with the confocal microscope -- Overview of laser microbeam applications as related to antibody targeting -- Nucleic acid immunocytochemistry: in situ hybridization to human chromosomes of an alkaline phosphatase-labeled centromeric probe -- **Fluorescence** in situ hybridization using whole chromosome **library** probes.
Overview of automated immunostainers -- Overview of immunocytochemical approaches to the differential diagnosis of tumors: diagnostic applications in the clinical laboratory.
PUB. COUNTRY: New Jersey; United States
DOCUMENT TYPE: Bibliography; (MONOGRAPH)
FILE SEGMENT: U.S. Imprints not USDA, Experiment or Extension
LANGUAGE: English

L5 ANSWER 25 OF 66 MEDLINE
ACCESSION NUMBER: 94253194 MEDLINE
DOCUMENT NUMBER: 94253194 PubMed ID: 8195250
TITLE: Expression cloning of a GM3-specific alpha-2,8-sialyltransferase (GD3 synthase).
AUTHOR: Sasaki K; Kurata K; Kojima N; Kurosawa N; Ohta S; Hanai N; Tsuji S; Nishi T
CORPORATE SOURCE: Tokyo Research Laboratories, Kyowa Hakko Kogyo, Co., Ltd., Japan.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1994 Jun 3) 269 (22) 15950-6.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-X77922
ENTRY MONTH: 199406
ENTRY DATE: Entered STN: 19940707
Last Updated on STN: 19980206
Entered Medline: 19940630
AB A cDNA encoding a GM3-specific alpha-2,8-sialyltransferase (GD3 synthase) was obtained from an expression cDNA **library** of human melanoma cell line WM266-4 by enrichment of Namalwa KJM-1 cells highly expressing GD3 using an anti-GD3 antibody and a **fluorescence**-activated cell sorter. Selection of B-cell line Namalwa cells expressing transfected cDNAs in the presence of anti-GD3 monoclonal antibody KM641 gave a cDNA (pAMo-GD3) encoding a protein with a type II transmembrane topology as found for mammalian glycosyltransferases. The following evidence confirms that the cDNA encodes an alpha-2,8-sialyltransferase, which specifically converts GM3 to GD3. (i) Transfection of pAMo-GD3 into Namalwa KJM-1 cells leads to the appearance of GD3 and a GD3 synthase activity. (ii) Northern blot analysis revealed a correlation between the expression of this gene and GD3 in several cell lines. (iii) The putative COOH-terminal active domain of this cloned enzyme fused with protein A has been purified with IgG-Sepharose beads and has been shown to possess GD3-synthesizing activity, excluding the possibility that the cloned cDNA encodes a transacting factor inducing a GD3 synthase. The deduced primary sequence also contains the "sialyl motif" conserved among all the sialyltransferases cloned to date. The polymerase chain reaction analysis reveals that this gene is located on chromosome 12.

L5 ANSWER 26 OF 66 MEDLINE DUPLICATE 8
ACCESSION NUMBER: 94286537 MEDLINE
DOCUMENT NUMBER: 94286537 PubMed ID: 8016078
TITLE: Pure chromosome-specific PCR libraries from single sorted chromosomes.
AUTHOR: VanDevanter D R; Choongkittaworn N M; Dyer K A; Aten J; Otto P; Behler C; Bryant E M; Rabinovitch P S

CORPORATE SOURCE: Tumor Institute, Swedish Medical Center, Seattle, WA 98104.
CONTRACT NUMBER: AG 01751 (NIA)
AG 10917 (NIA)
DK 32971 (NIDDK)
+
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1994 Jun 21) 91 (13) 5858-62.
Journal code: PV3; 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199407
ENTRY DATE: Entered STN: 19940810
Last Updated on STN: 19940810
Entered Medline: 19940728

AB Chromosome-specific DNA libraries can be very useful in molecular and cytogenetic genome mapping studies. We have developed a rapid and simple method for the generation of chromosome-specific DNA sequences that relies on polymerase chain reaction (PCR) amplification of a single flow-sorted chromosome or chromosome fragment. Previously reported methods for the development of chromosome libraries require larger numbers of chromosomes, with preparation of pure chromosomes sorted by **flow cytometry**, generation of somatic cell hybrids containing targeted chromosomes, or a combination of both procedures. These procedures are labor intensive, especially when hybrid cell lines are not already available, and this has limited the generation of chromosome-specific DNA libraries from nonhuman species. In contrast, a single sorted chromosome is a pure source of DNA for **library** production even when flow cytometric resolution of chromosome populations is poor. Furthermore, any sorting cytometer may be used with this technique. Using this approach, we demonstrate the generation of PCR libraries suitable for both molecular and **fluorescence** in situ hybridization studies from individual baboon and canine chromosomes, separate human homologues, and a rearranged marker chromosome from a transformed cell line. PCR libraries specific to subchromosomal regions have also been produced by sorting a small chromosome fragment. This simple and rapid technique will allow generation of nonhuman linkage maps and probes for **fluorescence** in situ hybridization and the characterization of marker chromosomes from solid tumors. In addition, allele-specific libraries generated by this strategy may also be useful for mapping genetic diseases.

L5 ANSWER 27 OF 66 MEDLINE
ACCESSION NUMBER: 94362249 MEDLINE
DOCUMENT NUMBER: 94362249 PubMed ID: 8080999
TITLE: Rh(D) antigen expression and isolation of a new Rh(D) cDNA isoform in human erythroleukemic K562 cells.
AUTHOR: Suyama K; Lunn R; Haller S; Goldstein J
CORPORATE SOURCE: Lindsley F. Kimball Research Institute, New York Blood Center, New York 10021.
SOURCE: BLOOD, (1994 Sep 15) 84 (6) 1975-81.
Journal code: A8G; 7603509. ISSN: 0006-4971.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
OTHER SOURCE: GENBANK-S73913
ENTRY MONTH: 199410
ENTRY DATE: Entered STN: 19941021
Last Updated on STN: 19970203
Entered Medline: 19941012

AB Human erythroleukemic K562 cells are known to have several erythroid properties. K562 cells possess Rh mRNAs, but expression of Rh proteins has not previously been reported. We immunoprecipitated Rh protein from K562 cell lysate using rabbit anti-Rh and detected Rh(D) antigens on K562 cells using **fluorescence**-activated cell sorting (FACS). These results suggest that K562 cells will be useful as an expression model for most Rh antigens. We also cloned a new Rh(D) cDNA isoform (RhK562-II), from a K562 cDNA **library** using polymerase chain reaction (PCR) with 5' and 3' end oligonucleotides of the published Rh(e/E) antigen encoding cDNA sequence as primers. Sequence analysis showed that RhK562-II is composed of 951 nucleotides (316 amino acids), identical to the first 939 nucleotides (exons 1 to 6) of one of the Rh(D) cDNAs (RhXIII), except for nucleotide 654 (C-->G exchange). However, this exchange is the same as that of another published Rh(D) cDNA (RhPII cDNA). RhK562-II is deprived of exons 7 and 8 (nucleotides 940 to 1,153), followed by an identical sequence up to the 3' end of the open-reading frame of the RhXIII cDNA, which causes a frame-shift mutation and produces a premature stop codon. In vitro expression of RhK562-II using the transcription and translation rabbit reticulocyte lysate system produced two major Rh-related proteins (30 kD and 25 kD), which were immunoprecipitated by rabbit polyclonal anti-Rh and separated on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

L5 ANSWER 28 OF 66 MEDLINE
ACCESSION NUMBER: 95048345 MEDLINE
DOCUMENT NUMBER: 95048345 PubMed ID: 7959744
TITLE: Generation of five high-complexity painting probe libraries from flow-sorted mouse chromosomes.
AUTHOR: Weier H U; Polikoff D; Fawcett J J; Greulich K M; Lee K H; Cram S; Chapman V M; Gray J W
CORPORATE SOURCE: Division of Molecular Cytometry, School of Medicine, University of California, San Francisco 94143-0808.
CONTRACT NUMBER: RR01315 (NCRR)
SOURCE: GENOMICS, (1994 Jun) 21 (3) 641-4.
Journal code: GEN; 8800135. ISSN: 0888-7543.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199411
ENTRY DATE: Entered STN: 19950110
Last Updated on STN: 19950110
Entered Medline: 19941130

AB Mouse metaphase chromosomes were purified by flow sorting from the murine fibroblast cell line Mus spretus clone 5A. We sorted chromosomes that fell into five individual peaks based on the Hoechst 33258/chromomycin A3 DNA histogram: three peaks corresponding to the least amount of DNA and two peaks representing chromosomes with the most DNA content. This is the first example of the successful application of bivariate flow karyotyping to murine chromosome sorting. We then applied primer-directed in vitro DNA amplification using the polymerase chain reaction (PCR) to generate and label larger amounts of chromosome-specific DNA. In situ hybridization showed specific binding of the PCR products to mouse chromosomes Y, 19, 18, 3, and X as well as chromosomes 1 and 2. The combination of chromosome sorting from the M. spretus cell line and PCR proved to be highly valuable for generation of pools of DNA fragments that exhibit specific binding to mouse chromosomes and can be used to identify and delineate mouse metaphase chromosomes.

L5 ANSWER 29 OF 66 MEDLINE
ACCESSION NUMBER: 94170829 MEDLINE
DOCUMENT NUMBER: 94170829 PubMed ID: 7510236
TITLE: MRC OX19 recognizes the rat CD5 surface glycoprotein, but does not provide evidence for a population of CD5bright B cells.
AUTHOR: Vermeer L A; de Boer N K; Bucci C; Bos N A; Kroese F G; Alberti S
CORPORATE SOURCE: Department of Histology and Cell Biology, University of Groningen, The Netherlands.
SOURCE: EUROPEAN JOURNAL OF IMMUNOLOGY, (1994 Mar) 24 (3) 585-92. Journal code: EN5; 1273201. ISSN: 0014-2980.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-X78985
ENTRY MONTH: 199404
ENTRY DATE: Entered STN: 19940420
Last Updated on STN: 19960129
Entered Medline: 19940412

AB To clone the rat CD5 gene we first produced two rat CD5 probes. The probes were obtained by polymerase chain reaction (PCR) on rat genomic DNA using primers designed on conserved regions between mouse and human CD5. The screening of a rat cDNA **library** at high stringency using these probes resulted in a 1.5-kb positive clone. The DNA sequence of this clone confirmed its CD5 nature, but the clone appeared to lack part of the 5' and part of the 3' end. These missing 5' and 3' ends were obtained by PCR on rat thymus RNA. By ligating these PCR products to the original 1.5-kb CDM8 clone, a full-length rat CD5 gene was constructed. The full-length clone showed high identity with mouse and human CD5; however, at the 5' site of the gene a region of 36 nucleotides is present which is not seen in either mouse or human CD5. We have evidence that this sequence is a normal constituent of the rat CD5 gene: first, it is in frame with the rest of the CD5 coding sequence; second, it does not contain a stop codon; and third, it is also present in the CD5 gene of other rat strains. We transfected the full-length CD5 construct in COS cells and demonstrated that indeed the CD5 protein is recognized by MRC OX19. Although we showed that CD5 mRNA is present in rat B cells, extensive **flow cytometry** analysis using MRC OX19 as antibody failed to detect B cells expressing significant levels of CD5 on their cell surface compared to other B cells in any tissue or cell suspension tested from a variety of rat strains. This is in contrast with the mouse where a distinct population of B cells (B-1a cells) can be found expressing more CD5 than the other B cells. Either B-1 cells are not present in rats or CD5 is not the right phenotypic marker for rat B-1 cells. It still remains to be investigated whether a population of B cells with functions similar to those of murine B-1 cells is present in rats.

L5 ANSWER 30 OF 66 MEDLINE
ACCESSION NUMBER: 94166767 MEDLINE
DOCUMENT NUMBER: 94166767 PubMed ID: 7907166
TITLE: Chromosome 2-specific DNA clones from flow-sorted chromosomes of tomato.
AUTHOR: Arumuganathan K; Martin G B; Telenius H; Tanksley S D; Earle E D
CORPORATE SOURCE: Department of Plant Breeding, Cornell University, Ithaca, NY 14853-1902.
SOURCE: MOLECULAR AND GENERAL GENETICS, (1994 Mar) 242 (5) 551-8. Journal code: NGP; 0125036. ISSN: 0026-8925.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199404
ENTRY DATE: Entered STN: 19940412
Last Updated on STN: 19950206
Entered Medline: 19940407

AB We obtained DNA clones specific to tomato chromosome 2 from a small number of chromosomes collected by flow sorting. Suspensions of metaphase chromosomes were prepared from 3-month-old tomato cell cultures of Lycopersicon pennellii. Isolated chromosomes stained with chromomycin A3 and Hoechst 33258 were analyzed on an EPICS 753 flow cytometer using a UV laser to excite Hoechst **fluorescence** and a 458 nm laser to excite chromomycin A3 **fluorescence**. Chromosomes from well-resolved peaks on a bivariate flow karyotype were sorted directly onto membrane filters for spot-blot analysis. The filters were processed and hybridized with chromosome-specific repetitive DNA probes. In this way tomato chromosome 1 and chromosome 2 were assigned to peaks in the bivariate flow karyotypes. One thousand copies of the putative chromosome 2 were flow-sorted directly into microfuge tubes. DNA specific to chromosome 2 was amplified by a polymerase chain reaction (PCR) technique using universal 22mer degenerate oligonucleotide primers (DOP) sequences. DOP-PCR yields a smear of fragments of various sizes from 250 to 1600 bp. Amplified products were cloned into the Bluescript plasmid vector. Approximately 11% of the clones contained sequences with highly repetitive elements, and 85% contained only low-copy-number sequences. Eleven clones containing low-copy-number sequences that detect restriction fragment

length polymorphisms were placed on the molecular linkage map of tomato. All showed linkage to chromosome 2.

L5 ANSWER 31 OF 66 MEDLINE
ACCESSION NUMBER: 94180407 MEDLINE
DOCUMENT NUMBER: 94180407 PubMed ID: 8133535
TITLE: Identification of partial complementary DNA clones encoding a 59-kd protein with characteristics of a unique oncofetal antigen.
AUTHOR: Rashid H U; Barsoum A L; Cao T M; Coggin J H Jr
CORPORATE SOURCE: Department of Microbiology and Immunology, University of South Alabama, College of Medicine, Mobile.
CONTRACT NUMBER: CA39698 (NCI)
SOURCE: JOURNAL OF THE NATIONAL CANCER INSTITUTE, (1994 Apr 6) 86 (7) 515-26.
Journal code: J9J; 7503089. ISSN: 0027-8874.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-U06662
ENTRY MONTH: 199404
ENTRY DATE: Entered STN: 19940428
Last Updated on STN: 19970203
Entered Medline: 19940420

AB BACKGROUND: Oncofetal antigens (OFAs) are conserved tumor-associated autoantigens or transplantation antigens present on the surface of all major classes of rodent and human tumors and on midgestational fetal cells but not on normal neonatal or adult human and rodent tissues. A syngeneically derived monoclonal antibody, MAb-115, recognizes murine OFAs of 44 and 200 kd in molecular mass. PURPOSE: Our goal was to clone and characterize the complementary DNAs (cDNAs) that encode these murine OFAs. METHODS: Rabbit antiserum raised against purified 44-kd OFA glycoprotein was used to screen a mouse embryo cDNA-lambda phage expression **library**. Recombinant phage clones positive for the expression of OFAs were detected by immunohistochemical staining, then isolated and plaque purified. The presence of an OFA-encoding sequence in the recombinant phage was confirmed by specific reaction of the expressed protein with MAb-115. Recombinant fusion protein was purified from the extracts of corresponding lysogens. Rabbit antiserum against purified recombinant fusion protein was raised, and the capacity of this antiserum to detect the expression of OFA on rodent tumor and fetal cells was determined by **flow cytometry**. In addition, immunoreactivity of tumor bearer and hyperimmune murine sera to bacterially expressed recombinant OFA protein was evaluated by enzyme-linked immunosorbent assay. The OFA-expressing insert DNA from plaque-purified lambda clones was subcloned into phagemid vectors for sequencing analysis. RESULTS: Antiserum derived against the isolated recombinant mouse embryo polypeptide mimicked MAb-115 in its specific binding to all OFA-positive rodent tumor and fetal cell lines tested and likewise did not show reactivity to normal adult tissues. This antiserum specifically recognized the native 44- and 200-kd OFAs in extracts of murine lymphocytic lymphoma. Furthermore, sera of tumor-bearing mice or mice immunized with purified OFA or intact, irradiated OFA-positive lymphocytic lymphoma cells also reacted with the recombinant fusion protein. The characterization of the isolated clone included nucleotide sequence information followed by analysis of the deduced primary structure of the protein. CONCLUSIONS: These data suggest that the isolated cDNA clones encode a distinct gene product which is widely expressed on the surface of tumor and fetal cells and represents the first characterized sequence of a true OFA. IMPLICATIONS: The availability of this cDNA, encoding a protein expressed only on tumor and fetal cells, provides a direct means to assess biological characteristics of malignant tissue which can be assayed by biochemical, histochemical, and molecular methods.

L5 ANSWER 32 OF 66 MEDLINE DUPLICATE 9
ACCESSION NUMBER: 94359457 MEDLINE
DOCUMENT NUMBER: 94359457 PubMed ID: 8078460
TITLE: Genome analysis of the moss Physcomitrella patens (Hedw.) B.S.G.
AUTHOR: Reski R; Faust M; Wang X H; Wehe M; Abel W O
CORPORATE SOURCE: Institut fur Allgemeine Botanik, Hamburg, Germany.
SOURCE: MOLECULAR AND GENERAL GENETICS, (1994 Aug 15) 244 (4) 352-9.
Journal code: NGP; 0125036. ISSN: 0026-8925.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-X76632; GENBANK-X76633; GENBANK-X76634
ENTRY MONTH: 199410
ENTRY DATE: Entered STN: 19941013
Last Updated on STN: 19980206
Entered Medline: 19941006

AB A wild-type (WT) strain of the moss Physcomitrella patens (Hedw.) B.S.G., two mutants derived from it (PC22 and P24), and a somatic hybrid, PC22(+)P24, were analysed. Staining of metaphases revealed 54 +/- 2 chromosomes in the somatic hybrid and 27 chromosomes in the wild type and the two mutants. Using **flow cytometry** (FCM), DNA contents were calculated to be 0.6 pg (WT, PC22), 1.2 pg (P24), and 1.6 pg (PC22(+)P24) per nucleus, respectively. Southern hybridization provided evidence for at least one family of highly repetitive DNA and, furthermore, revealed different amounts of repetitive DNA in the four genotypes. However, these sequences cannot account for the 100% increase in the nuclear DNA amount in mutant P24, relative to wild type. In FCM analyses every moss genotype generated just one single peak of **fluorescence**, indicating an arrest in the cell cycle during the daytime. Thermal denaturation of wild-type DNA revealed a G+C content of 34.6% for total DNA and 38.6% for plastid DNA. A cDNA **library** of 1.2 x 10(6) independent clones was established, from which sequences homologous to cab and rbcS, respectively, were isolated. These genes show significant homologies to those of higher plants, and, likewise, comprise multigene families. No restriction fragment length polymorphisms could be detected between the four moss genotypes using these cDNA probes.

L5 ANSWER 33 OF 66 MEDLINE
ACCESSION NUMBER: 94305578 MEDLINE
DOCUMENT NUMBER: 94305578 PubMed ID: 7518285
TITLE: Chromosome sorting by **flow cytometry**. Production of DNA libraries and gene mapping.
AUTHOR: Fantes J A; Green D K; Sharkey A
CORPORATE SOURCE: Human Genetics Unit, Western General Hospital, Edinburgh, Scotland.
SOURCE: METHODS IN MOLECULAR BIOLOGY, (1994) 29 205-19. Journal code: BU3; 9214969. ISSN: 1064-3745.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199408
ENTRY DATE: Entered STN: 19940825
Last Updated on STN: 19960129
Entered Medline: 19940816

L5 ANSWER 34 OF 66 MEDLINE DUPLICATE 10
ACCESSION NUMBER: 94101880 MEDLINE
DOCUMENT NUMBER: 94101880 PubMed ID: 8275710
TITLE: A mouse chromosome 11 **library** generated from sorted chromosomes using linker-adapter polymerase chain reaction.
AUTHOR: Miyashita K; Vooijs M A; Tucker J D; Lee D A; Gray J W; Pallavicini M G
CORPORATE SOURCE: Department of Laboratory Medicine, University of California, San Francisco 94103.
CONTRACT NUMBER: 27703
SOURCE: CYTOGENETICS AND CELL GENETICS, (1994) 66 (1) 54-7. Journal code: DXK; 0367735. ISSN: 0301-0171.
PUB. COUNTRY: Switzerland
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199402
ENTRY DATE: Entered STN: 19940218
Last Updated on STN: 19940218
Entered Medline: 19940204
AB We describe the generation of a mouse whole chromosome **library** using sequence-independent polymerase chain reaction (PCR) to amplify sequences contained in DNA extracted from flow sorted chromosomes. DNA in sorted chromosomes from a human x mouse hybrid cell line was digested with a frequent four-cutter restriction enzyme, Sau3AI, and the ends were ligated to an adapter oligonucleotide. The ligated DNA fragments were amplified using PCR primers homologous to the linker-adapter oligonucleotide. PCR-generated products were characterized by gel electrophoresis. The size of the amplified DNA ranged from 100 to more than 1,000 bp with a relatively high proportion of products at approximately 400 bp. Biotinylated PCR products used for FISH showed specific hybridization to murine metaphases and no hybridization to human lymphocyte and hamster metaphase cells. Banding analysis indicated that the probes were specific for mouse Chromosome 11. We anticipate that availability of painting probes for specific murine chromosomes will facilitate cytogenetic studies in the mouse.

L5 ANSWER 35 OF 66 MEDLINE
ACCESSION NUMBER: 93352474 MEDLINE
DOCUMENT NUMBER: 93352474 PubMed ID: 8394326
TITLE: Human aminoacylase-1. Cloning, sequence, and expression analysis of a chromosome 3p21 gene inactivated in small cell lung cancer.
AUTHOR: Cook R M; Burke B J; Buchhagen D L; Minna J D; Miller Y E
CORPORATE SOURCE: Division of Pulmonary Sciences and Critical Care Medicine, University of Colorado Health Sciences Center, Denver 80262.
CONTRACT NUMBER: HL-07085 (NHLBI)
P50 CA58187 (NCI)
RO1 HL45745 (NHLBI)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1993 Aug 15) 268 (23) 17010-7. Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-L07548
ENTRY MONTH: 199309
ENTRY DATE: Entered STN: 19931001
Last Updated on STN: 19970203
Entered Medline: 19930915
AB Human aminoacylase-1 (N-acyl-L-amino-acid amidohydrolase, EC 3.5.1.14; ACY1) is a homodimeric zinc-binding enzyme that catalyzes the hydrolysis of N alpha-acylated amino acids. ACY1 has been assigned to chromosome 3p21.1, a region reduced to homozygosity in small cell lung cancer (SCLC), and has been reported to exhibit reduced or absent expression in SCLC cell lines and tumors. Two human cDNA libraries and one human genomic DNA **library** were screened with a previously isolated partial ACY1 cDNA to isolate a full-length transcript. Sequence analysis of clones from each of these libraries resulted in an ACY1 cDNA of 1438 base pairs with an open reading frame of 1224-base pairs coding for a putative protein of 408 amino acids with a predicted molecular mass of 45,882 Da. Sequence analysis revealed no homologies to previously reported cDNA or protein sequences and establishes ACY1 as the first member of a new family of zinc-binding enzymes to be so characterized. The subcellular location of ACY1 has been established as cytosolic by **flow cytometry** . Southern and northern analyses of ACY1 in SCLC cell lines failed to demonstrate any gross abnormalities of the ACY1 structural gene or instances of absent or aberrantly sized mRNA, respectively.

L5 ANSWER 36 OF 66 MEDLINE DUPLICATE 11
ACCESSION NUMBER: 94068469 MEDLINE

DOCUMENT NUMBER: 94068469 PubMed ID: 7504279
TITLE: Generation and screening of an oligonucleotide-encoded synthetic peptide **library**.
AUTHOR: Needels M C; Jones D G; Tate E H; Heinkel G L; Kochersperger L M; Dower W J; Barrett R W; Gallop M A
CORPORATE SOURCE: Affymax Research Institute, Palo Alto, CA 94304.
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1993 Nov 15) 90 (22) 10700-4. Journal code: PV3; 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199312
ENTRY DATE: Entered STN: 19940201
Last Updated on STN: 19960129
Entered Medline: 19931229

AB We have prepared a **library** of approximately 10(6) different peptide sequences on small, spherical (10-microns diameter) beads by the combinatorial chemical coupling of both L- and D-amino acid building blocks. To each bead is covalently attached many copies of a single peptide sequence and, additionally, copies of a unique single-stranded oligonucleotide that codes for that peptide sequence. The oligonucleotide tags are synthesized through a parallel combinatorial procedure that effectively records the process by which the encoded peptide sequence is assembled. The collection of beads was screened for binding to a fluorescently labeled anti-peptide antibody using a **fluorescence** -activated cell sorting instrument. Those beads to which the antibody bound tightly were isolated by **fluorescence**-activated sorting, and the oligonucleotide identifiers attached to individual sorted beads were amplified by the PCR. Sequences of the amplified DNAs were determined to reveal the identity of peptide sequences that bound to the antibody with high affinity. By combining the capacity for information storage in an oligonucleotide code with the tremendous level of amplification possible through the PCR, we have devised a means for specifying the identity of each member of a vast **library** of molecules synthesized from both natural and unnatural chemical building blocks. In addition, we have shown that the use of **flow cytometry** instrumentation permits facile isolation of individual beads that bear high-affinity ligands for biological receptors.

L5 ANSWER 37 OF 66 MEDLINE
ACCESSION NUMBER: 94077713 MEDLINE
DOCUMENT NUMBER: 94077713 PubMed ID: 8255765
TITLE: Coincidence painting: a rapid method for cloning region specific DNA sequences.
COMMENT: Erratum in: Nucleic Acids Res 1994 Feb 25;22(4):700
AUTHOR: Bailey D M; Carter N P; de Vos D; Leversha M A; Perryman M T; Ferguson-Smith M A
CORPORATE SOURCE: Department of Pathology, Cambridge University, UK.
SOURCE: NUCLEIC ACIDS RESEARCH, (1993 Nov 11) 21 (22) 5117-23. Journal code: O8L; 0411011. ISSN: 0305-1048.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199401
ENTRY DATE: Entered STN: 19940203
Last Updated on STN: 19950206
Entered Medline: 19940113

AB We have developed a novel coincidence cloning strategy, termed Coincidence Painting, which enables the rapid generation of large numbers of region specific sequences. Coincidence Painting utilises Degenerate Oligonucleotide Primed PCR (DOP-PCR) amplification of flow sorted derivative translocation chromosomes. The PCR products are hybridised in situ onto specific flow sorted chromosomes for coincident sequence selection. Eluted and reamplified material is then cloned using a novel insert end revelation and ligation technique. Cloned inserts range in size from 150-1300 bps of which approximately 54% appear to be single copy sequences. The cloning method permits the excision of vector free probe for **library** hybridisation screening and the small insert size facilitates analysis for the generation of sequence tagged sites (STSs). We have used such clones successfully for YAC screening by PCR and for cosmid screening by filter hybridisation. This new methodology should allow the rapid saturation with probes of regions defined by specific translocation breakpoints.

L5 ANSWER 38 OF 66 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 93356822 EMBASE
DOCUMENT NUMBER: 1993356822
TITLE: A strategy for the characterization of minute chromosome rearrangements using multiple color **fluorescence** in situ hybridization with chromosome-specific DNA libraries and YAC clones.
AUTHOR: Popp S.; Jauch A.; Schindler D.; Speicher M.R.; Lengauer C.; Donis-Keller H.; Riethman H.C.; Cremer T.
CORPORATE SOURCE: Inst Humangenetik und Anthropologie, Universitat Heidelberg, Im Neuenheimer Feld 328,D-69120 Heidelberg, Germany
SOURCE: Human Genetics, (1993) 92/6 (527-532). ISSN: 0340-6717 CODEN: HUGEDQ
COUNTRY: Germany
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 022 Human Genetics
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The identification of marker chromosomes in clinical and tumor cytogenetics by chromosome banding analysis can create problems. In this study, we present a strategy to define minute chromosomal rearrangements by multicolor **fluorescence** in situ hybridization (FISH) with 'whole chromosome painting' probes derived from chromosome-specific DNA libraries and Alu-polymerase chain reaction (PCR) products of various region-specific yeast artificial chromosome (YAC) clones. To demonstrate the usefulness of this strategy for the characterization of chromosome

rearrangements unidentifiable by banding techniques, an 8p+ marker chromosome with two extra bands present in the karyotype of a child with multiple anomalies, malformations, and severe mental retardation was investigated. A series of seven-color FISH experiments with sets of fluorochrome-labeled DNA **library** probes from flow-sorted chromosomes demonstrated that the additional segment on 8p+ was derived from chromosome 6. For a more detailed characterization of the marker chromosome, three-color FISH experiments with **library** probes specific to chromosomes 6 and 8 were performed in combination with newly established telomeric and subtelomeric YAC clones from 6q25, 6p23, and 8p23. These experiments demonstrated a trisomy 6pter.fwdarw.6p22 and a monosomy 8pter.fwdarw.8p23 in the patient. The present limitations for a broad application of this strategy and its possible improvements are discussed.

L5 ANSWER 39 OF 66 MEDLINE
ACCESSION NUMBER: 94172908 MEDLINE
DOCUMENT NUMBER: 94172908 PubMed ID: 8126985
TITLE: Human gene mapping and chromosome sorting by laser technology.
AUTHOR: Shimizu N; Minoshima S; Kudoh J; Kawasaki K; Wang Y; Shimizu Y; Sakai K
CORPORATE SOURCE: Department of Molecular Biology, School of Medicine, Keio University, Tokyo, Japan.
SOURCE: KEIO JOURNAL OF MEDICINE, (1993 Dec) 42 (4) 212-6. Journal code: KUJ; 0376354. ISSN: 0022-9717.
PUB. COUNTRY: Japan
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199404
ENTRY DATE: Entered STN: 19940420
Last Updated on STN: 19940420
Entered Medline: 19940408
AB More than 30 human genes have been mapped by a combination of the **fluorescent** in situ hybridization (FISH) and spot blot hybridization in this laboratory for the past five years. Furthermore, the Keio strategy has been established for the molecular analysis of the human genome using flow-sorted human chromosomes.

L5 ANSWER 40 OF 66 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 93040892 EMBASE
DOCUMENT NUMBER: 1993040892
TITLE: Refined examination of plant metaphase chromosome structure at different levels made feasible by new isolation methods.
AUTHOR: Schubert I.; Dolezel J.; Houben A.; Scherthan H.; Wanner G.
CORPORATE SOURCE: Institute of Plant Genetics, Crop Plant Research,O-4325 Gatersleben, Germany
SOURCE: Chromosoma, (1993) 102/2 (96-101). ISSN: 0009-5915 CODEN: CHROAU
COUNTRY: Germany
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 022 Human Genetics
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Two methods for isolation of plant metaphase chromosomes are described. The first, micromanipulation, allows the isolation of a number of individual chromosomes, which may be used as templates for the generation of chromosome specific DNA libraries and for physical sequence mapping by the polymerase chain reaction (PCR). The second provides, from synchronized meristems, pure chromosome suspensions suitable for flow cytometric analysis and chromosome sorting. Restriction endonuclease banding, immunostaining of chromosomal antigens, as well as **fluorescence** in situ hybridization at high signal to noise ratio were successfully performed on the isolated chromosomes. Chromosomes obtained by both protocols were suitable for scanning electron microscopy, the methods should also prove useful for refined analyses of the karyotypes of other plant species.

L5 ANSWER 41 OF 66 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 93047361 EMBASE
DOCUMENT NUMBER: 1993047361
TITLE: Identification of a tumor marker chromosome by flow sorting, DNA amplification in vitro, and in situ hybridization of the amplified product.
AUTHOR: Boschman G.A.; Buys C.H.C.M.; Van der Veen A.Y.; Rens W.; Osinga J.; Slater R.M.; Aten J.A.
CORPORATE SOURCE: Academisch Medisch Centrum F0-212, Meibergdreef 9,1105 AZ Amsterdam, Netherlands
SOURCE: Genes Chromosomes and Cancer, (1993) 6/1 (10-16). ISSN: 1045-2257 CODEN: GCCAES
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 016 Cancer
022 Human Genetics
LANGUAGE: English
SUMMARY LANGUAGE: English
AB A method combining flow sorting and molecular cytogenetic techniques for the identification of unknown marker chromosomes is described. In this study, the bladder tumor cell line J82 was used, which was known to carry a marker chromosome of the size of chromosome 7 in every cell. From the cytogenetic analysis of Q-banded metaphase cells, it was shown to be composed of .apprx.40% presumably the greater part of chromosome 20 and for the rest microscopically unidentifiable material. This marker chromosome was found using flow cytometric analysis to form an independent peak and hence was suitable for isolation using dual-parameter sorting after staining with Hoechst 33258 and chromomycin A3. Subsequently, the marker was isolated by dual-parameter sorting. DNA amplification of 300 isolated chromosomes by polymerase chain reaction (PCR) using the Alu-primer Bk33 and the LINES-primer LH5 was carried out. After purification of the amplified product, a yield of 5 .mu.m of DNA was obtained. The DNA was labelled using Bio-II-dUTP and applied to human lymphocyte metaphase cells in a suppressive in situ hybridization procedure. **Fluorescence** was visible over chromosome 20 and over

the distal one-half of 6p. Together the **fluorescent** regions accounted for only .apprx.60% of the marker length, indicating a possible duplication of chromosome 20 material. This was confirmed by applying bicolor in situ hybridization using chromosome 6- and 20-specific DNA libraries to metaphase cells of the J82 cells.

L5 ANSWER 42 OF 66 MEDLINE
ACCESSION NUMBER: 94001757 MEDLINE
DOCUMENT NUMBER: 94001757 PubMed ID: 7691162
TITLE: Chromosomal radiosensitivity at intrachromosomal telomeric sites.
AUTHOR: Alvarez L; Evans J W; Wilks R; Lucas J N; Brown J M; Giaccia A J
CORPORATE SOURCE: Stanford University School of Medicine, Cancer Biology Research Laboratory, CA 94305-5468.
CONTRACT NUMBER: CA 55801 (NCI)
CA 58838 (NCI)
SOURCE: GENES, CHROMOSOMES AND CANCER, (1993 Sep) 8 (1) 8-14.
Journal code: AYV; 9007329. ISSN: 1045-2257.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199311
ENTRY DATE: Entered STN: 19940117
Last Updated on STN: 19960129
Entered Medline: 19931119

AB Telomeric DNA is composed of highly conserved sequences which are present at the termini of chromosomes as well as at intrachromosomal locations. Here, we studied a Chinese hamster ovary (CHO) cell line, BL-10, with highly stable amplified telomeric DNA at the termini as well as at intrachromosomal locations. We show that intrachromosomal or interstitial telomeric sites in this cell line and in another CHO cell line, HA-I, are radiosensitive in that they are more prone to breakage than would be expected based on the percentage of the genome composed of telomeric sequences. The frequency of breakage at interstitial telomeric sites is 4.3 to 8.3 times higher than that in the CHO genome overall. These conclusions are reached by both conventional cytogenetic analysis of two CHO cell lines which have the same survival rates after exposure to ionizing radiation, and by use of double **fluorescence** in situ hybridization (FISH) with a pan-telomere-specific probe and a CHO chromosome-specific **library** in the same metaphase cells after irradiation.

L5 ANSWER 43 OF 66 MEDLINE
ACCESSION NUMBER: 92302264 MEDLINE
DOCUMENT NUMBER: 92302264 PubMed ID: 1376921
TITLE: Random PCR mutagenesis screening of secreted proteins by direct expression in mammalian cells.
AUTHOR: Rice G C; Goeddel D V; Cachianes G; Woronicz J; Chen E Y; Williams S R; Leung D W
CORPORATE SOURCE: Department of Cell Biology, Genentech, Inc., South San Francisco, CA 94080.
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1992 Jun 15) 89 (12) 5467-71.
Journal code: PV3; 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199207
ENTRY DATE: Entered STN: 19920731
Last Updated on STN: 19980206
Entered Medline: 19920721

AB We have developed a general method for screening randomly mutagenized expression libraries in mammalian cells by using **fluorescence** -activated cell sorting (FACS). The cDNA sequence of a secreted protein is randomly mutagenized by PCR under conditions of reduced Taq polymerase fidelity. The mutated DNA is inserted into an expression vector encoding the membrane glycophospholipid anchor sequence of decay-accelerating factor (DAF) fused to the C terminus of the secreted protein. This results in expression of the protein on the cell surface in transiently transfected mammalian cells, which can then be screened by FACS. This method was used to isolate mutants in the kringle 1 (K1) domain of tissue plasminogen activator (t-PA) that would no longer be recognized by a specific monoclonal antibody (mAb387) that inhibits binding of t-PA to its clearance receptor. DNA sequence analysis of the mutants and localization of the mutated residues on a three-dimensional model of the K1 domain identified three key discontinuous amino acid residues that are essential for mAb387 binding. Mutants with changes in any of these three residues were found to have reduced binding to the t-PA receptor on human hepatoma HepG2 cells but to retain full clot lysis activity.

L5 ANSWER 44 OF 66 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 92292171 EMBASE
DOCUMENT NUMBER: 1992292171
TITLE: Structural chromosome 1 aberrations in transitional cell carcinoma of the bladder: Interphase cytogenetics combining a centromeric, telomeric, and **library** DNA probe.
AUTHOR: Poddighe P.J.; Ramaekers F.C.S.; Smeets A.W.G.B.; Vooijs G.P.; Hopman A.H.N.
CORPORATE SOURCE: Molecular Cell Biol./Genetics Dept., University of Limburg, P.O. Box 616,6200 MD Maastricht, Netherlands
SOURCE: Cancer Research, (1992) 52/18 (4929-4934).
ISSN: 0008-5472 CODEN: CNREA8
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 016 Cancer
022 Human Genetics
028 Urology and Nephrology
LANGUAGE: English
SUMMARY LANGUAGE: English

AB **Fluorescence** in situ hybridization (FISH) was used to study numerical and structural chromosome 1 aberrations in interphase nuclei of

transitional cell carcinomas (TCCs) of the urinary bladder. One of the characteristic numerical aberrations, as detected previously in low-grade noninvasive TCCs, included trisomy for chromosome 1 (A. H. N. Hopman et al., Cancer Res., 51: 644-651, 1991). We examined in more detail 22 cases with a centromeric (1q12) and a telomeric associated (1p36) DNA probe and with a **library** DNA probe from sorted human chromosome 1 in single- and double-target FISH procedures. All flow cytometrically determined DNA diploid TCCs (13 cases), which showed three spots for 1q12 (6 cases), had two spots for 1p36. Since the **library** DNA probe showed three separate domains in the nuclei of these cases, the additional copy for 1q12 could be explained as an extra chromosome 1p-, containing the 1q12 target. In the flow cytometrically determined DNA tetraploid/aneuploid tumors, the results were more complex. In 6 of 9 cases, we observed an overrepresentation of 1q12 as compared to 1p36, also suggesting the presence of extra copies of 1p- chromosomes. The results of the present study demonstrate the utility of the FISH method to assess structural chromosome aberrations in interphase nuclei of solid tumors.

L5 ANSWER 45 OF 66 MEDLINE
ACCESSION NUMBER: 92228814 MEDLINE
DOCUMENT NUMBER: 92228814 PubMed ID: 1565644
TITLE: Cloning and expression of the tumor-associated antigen L6.
AUTHOR: Marken J S; Schieven G L; Hellstrom I; Hellstrom K E; Aruffo A
CORPORATE SOURCE: Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, WA 98121.
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1992 Apr 15) 89 (8) 3503-7. Journal code: PV3; 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-M90657
ENTRY MONTH: 199205
ENTRY DATE: Entered STN: 19920607
Last Updated on STN: 19950206
Entered Medline: 19920515

AB The L6 cell surface antigen, which is highly expressed on lung, breast, colon, and ovarian carcinomas, has attracted attention as a therapeutic target for murine monoclonal antibodies and their humanized counterparts. Its molecular nature has, however, remained elusive. Here we describe the expression cloning of a cDNA encoding the L6 antigen. COS cells transfected with this cDNA direct the expression of an approximately 24-kDa surface protein that reacts with the two anti-L6 monoclonal antibodies available. The predicted L6 peptide sequence is 202 amino acids long and contains three predicted NH2-terminal hydrophobic transmembrane regions, which are followed by a hydrophilic region containing two potential N-linked glycosylation sites and a COOH-terminal hydrophobic transmembrane region. The L6 antigen is related to a number of cell surface proteins with similar predicted membrane topology that have been implicated in cell growth. Two other members of this family of proteins, CD63 (ME491) and CO-029, are also highly expressed on tumor cells. The present findings should make it possible to further study the role of the L6-defined antigen in normal and neoplastic cells and to construct animal models for development of improved agents for active and passive cancer immunotherapy.

L5 ANSWER 46 OF 66 MEDLINE
ACCESSION NUMBER: 92242909 MEDLINE
DOCUMENT NUMBER: 92242909 PubMed ID: 1573270
TITLE: A member of the tetra spans transmembrane protein superfamily is recognized by a monoclonal antibody raised against an HLA class I-deficient, lymphokine-activated killer-susceptible, B lymphocyte line. Cloning and preliminary functional studies.
AUTHOR: Gil M L; Vita N; Lebel-Binay S; Miloux B; Chalon P; Kaghad M; Marchiol-Fournigault C; Conjeaud H; Caput D; Ferrara P; +
CORPORATE SOURCE: UA1156 Centre National de la Recherche Scientifique, Institut Gustave Roussy, Villejuif, France.
SOURCE: JOURNAL OF IMMUNOLOGY, (1992 May 1) 148 (9) 2826-33. Journal code: IFB; 2985117R. ISSN: 0022-1767.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 199206
ENTRY DATE: Entered STN: 19920619
Last Updated on STN: 19980206
Entered Medline: 19920602

AB The IA4 mAb was identified among a series of antibodies raised in BALB/c mice after immunization against a HLA class I-deficient, lymphokine-activated killer (LAK)-susceptible EBV-B lymphocyte line. The IA4 antibody was selected because of its high expression, in the range of 10(5) to 25 x 10(5) sites/cell, on several B lymphocyte lines (EBV-transformed or Burkitt) and monocytic lines such as HL60 and U937, and because its expression was correlated with both target susceptibility to LAK lysis and reduced expression of HLA class I surface Ag on two pairs of EBV-B-transformed cell lines (721/721.134 and MM/10F2). Despite the strategy followed to raise the mAb and the correlation mentioned above, no direct role of the IA4 molecules in LAK susceptibility has been established, since the IA4 molecule is poorly expressed on the sensitive targets Daudi and K562; moreover, the IA4 antibody did not affect reproducibly the in vitro killing of positive target cells by LAK effectors. The IA4 antibody was poorly immunoprecipitating and the surface molecule recognized was identified by gene cloning following an expression strategy using a U937 cDNA **library** transfected in COS cells, and a screening strategy based on membrane expression of IA4 molecule. The IA4 cDNA is virtually identical to "R2," a mRNA species previously identified in activated human T cells by subtractive hybridization. The IA4 cDNA contains an open reading frame coding for a protein 267 amino acids long with four potential transmembrane domains and one large external hydrophilic domain of about 110 amino acids, possibly glycosylated. The

encoded protein belongs to a family of surface molecules, the tetra spans transmembrane protein superfamily, all displaying the four transmembrane domains, expressed on various cell types including lymphocytes (CD9, CD37, CD53, TAPA-1), melanoma cells (ME491), and intestinal cells (CO-029). These molecules have been reported to be involved in cell activation and cell death. Surprisingly, the Schistosoma mansoni Ag Sm23 displays significant homologies with this family. The IA4 molecule is a widely distributed surface marker expressed on circulating lymphocytes and monocytes, newborn thymocytes, and the cell lines mentioned above. The IA4 molecule expression is up-regulated upon cell activation. Weakly expressed on resting peripheral T and B lymphocytes and large granular lymphocytes (NK), its expression roughly doubles after activation by PHA, staphylococcus aureus Cowan I, and IL-2, respectively.(ABSTRACT TRUNCATED AT 400 WORDS)

L5 ANSWER 47 OF 66 MEDLINE
ACCESSION NUMBER: 93078307 MEDLINE
DOCUMENT NUMBER: 93078307 PubMed ID: 1447820
TITLE: Flow karyotyping and chromosome sorting.
AUTHOR: Hashimoto K
CORPORATE SOURCE: Division of Genetic Resources, National Institute of Health.
SOURCE: NIPPON RINSHO. JAPANESE JOURNAL OF CLINICAL MEDICINE, (1992 Oct) 50 (10) 2484-8. Ref: 9
Journal code: KIM; 0420546. ISSN: 0047-1852.
PUB. COUNTRY: Japan
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW LITERATURE)
LANGUAGE: Japanese
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199212
ENTRY DATE: Entered STN: 19930129
Last Updated on STN: 19930129
Entered Medline: 19921229
AB For flow karyotyping and sorting, chromosomes are stained in suspension with appropriate DNA fluorochromes, and passed singly through a beam of laser light. The emitted pulses of **fluorescence** and scattered light are measured and stored in the form of a histogram displaying the number of pulses versus **fluorescence** or scattered light intensity. A data processing system was devised to remove signals from cell debris or chromosome fragments. A selective window was set in a dot plot of **fluorescence** versus scattered light intensities and a program was made to count pulses from the gated area only. As application of chromosome fractionation, **flow cytometry** has been used for karyotype analysis, gene mapping, and chromosomal DNA **library** construction.

L5 ANSWER 48 OF 66 MEDLINE DUPLICATE 12
ACCESSION NUMBER: 92347875 MEDLINE
DOCUMENT NUMBER: 92347875 PubMed ID: 1639403
TITLE: Molecular mapping of mouse chromosomes 4 and 6: use of a flow-sorted Robertsonian chromosome.
AUTHOR: Bahary N; Pachter J E; Felman R; Leibel R L; Albright K; Cram S; Friedman J M
CORPORATE SOURCE: Howard Hughes Medical Institute, Rockefeller University, New York, New York 10021.
CONTRACT NUMBER: DK26687 (NIDDK)
DK41096 (NIDDK)
HG00316 (NHGRI)
SOURCE: GENOMICS, (1992 Jul) 13 (3) 761-9.
Journal code: GEN; 8800135. ISSN: 0888-7543.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199208
ENTRY DATE: Entered STN: 19920911
Last Updated on STN: 19980206
Entered Medline: 19920828
AB The development of dense genetic maps of mammalian chromosomes is facilitated when chromosome-specific libraries are used as a source of genetic markers. To saturate the genetic maps of mouse chromosomes 4 and 6, we have made use of **fluorescent**-activated chromosome sorting to purify a 4:6 Robertsonian chromosome from a cell line harboring the Rb(4:6)2Bnr translocation. After staining with chromomycin A3 and Hoechst 33528, this chromosome was separated from the other mouse chromosomes. DNA was isolated from the fraction containing the Robertsonian chromosome and subcloned into the insertion vector lambda gt10, generating a **library** with 4.6 x 10(5) independent phage. A total of 19 single-copy sequences were used to type the progeny of a C57BL/6J x Mus spretus backcross that had previously been typed for loci on chromosomes 4 and 6. Approximately 70% of the clones in the **library** mapped to either chromosome 4 or 6 as assessed by genetic mapping and by use of a somatic cell hybrid panel. Simple sequence repeats have also been isolated from this **library**. Further characterization of these microsatellites should accelerate efforts to map mouse chromosomes 4 and 6 using PCR. In addition, flow sorting of Robertsonian chromosomes suggests a general approach for making chromosome-specific libraries in mouse.

L5 ANSWER 49 OF 66 MEDLINE DUPLICATE 13
ACCESSION NUMBER: 93052373 MEDLINE
DOCUMENT NUMBER: 93052373 PubMed ID: 1427877
TITLE: Mapping of 50 cosmid clones isolated from a flow-sorted human X chromosome **library** by **fluorescence** in situ hybridization.
AUTHOR: Fan Y S; Sasi R; Lee C; Court D; Lin C C
CORPORATE SOURCE: Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, Canada.
SOURCE: GENOMICS, (1992 Oct) 14 (2) 542-5.
Journal code: GEN; 8800135. ISSN: 0888-7543.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English

FILE SEGMENT: Priority Journals
ENTRY MONTH: 199212
ENTRY DATE: Entered STN: 19930122
Last Updated on STN: 19930122
Entered Medline: 19921201

AB Fifty cosmids have been mapped to metaphase chromosomes by **fluorescence** in situ hybridization under conditions that suppress signals from repetitive DNA sequences. The cosmid clones were isolated from a flow-sorted human X chromosome **library**. Thirty-eight of the clones were localized to chromosome X and 12 to autosomes such as chromosomes 3, 7, 8, 14, and 17. Although most of the cosmids mapped to the X chromosome appeared to be scattered along both the short and long arms, 10 cosmids were localized to the centromeric region of the chromosome. Southern blot analysis revealed that only two of these clones hybridized to probe pXBR-1, which detects the DXZ1 locus. In addition, 4 out of 5 cosmids mapped on chromosome 8 also localized on the centromeric region. While localization of X-specific cosmids will facilitate the physical mapping of the human X chromosome, cosmids mapped to the centromeric regions of chromosomes X and 8 should be especially useful for studying the structure and organization of these regions.

L5 ANSWER 50 OF 66 MEDLINE
ACCESSION NUMBER: 93131264 MEDLINE
DOCUMENT NUMBER: 93131264 PubMed ID: 1483693
TITLE: Complete characterization of a large marker chromosome by reverse and forward chromosome painting.
AUTHOR: Blennow E; Telenius H; Larsson C; de Vos D; Bajalica S; Ponder B A; Nordenskjold M
CORPORATE SOURCE: Department of Clinical Genetics, Karolinska Institute, Stockholm, Sweden.
SOURCE: HUMAN GENETICS, (1992 Dec) 90 (4) 371-4.
Journal code: GED; 7613873. ISSN: 0340-6717.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199302
ENTRY DATE: Entered STN: 19930226
Last Updated on STN: 19930226
Entered Medline: 19930218

AB Marker chromosome are small supernumerary chromosomes that are sometimes associated with developmental abnormalities. Hence, the genes involved in such cases provide an interesting approach to understanding developmental abnormalities in man. As a first step towards isolating such sequences, marker chromosomes need complete characterization. By combining chromosome isolation by flow sorting and the "degenerate oligonucleotide primed - polymerase chain reaction", we have constructed a DNA **library** specific for a marker chromosome found in a child with severe developmental abnormalities. We used **fluorescent** in situ hybridization of the **library** onto normal metaphase spreads ("reverse chromosome painting") and were thus able to determine that the marker consists of the centromeric part of chromosome 7, the telomeric region of the long arm of chromosome 5 and the telomeric region of the short arm of the X-chromosome. Subsequently, we hybridized normal chromosome-specific libraries of the relevant chromosomes onto metaphases containing the marker chromosome ("forward chromosome painting") and could in this manner establish the precise location of the different chromosome regions on the marker chromosome itself. This is a general approach suitable for outlining marker chromosomes in detail, and will aid the identification of the genes involved.

L5 ANSWER 51 OF 66 MEDLINE
ACCESSION NUMBER: 92175525 MEDLINE
DOCUMENT NUMBER: 92175525 PubMed ID: 1311700
TITLE: Isolation of a clone which induces expression of the gene encoding the human tumor necrosis factor receptor.
AUTHOR: Garret M; McHendry-Rinde B; Spickofsky N; Margolskee R F
CORPORATE SOURCE: Department of Neurosciences, Roche Research Center, Nutley, NJ 07110.
SOURCE: GENE, (1992 Feb 15) 111 (2) 215-22.
Journal code: FOP; 7706761. ISSN: 0378-1119.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-M64367; GENBANK-M64425; GENBANK-M64426; GENBANK-M64427; GENBANK-M85145; GENBANK-S39996; GENBANK-S40001; GENBANK-S40008; GENBANK-S40009; GENBANK-S40010
ENTRY MONTH: 199204
ENTRY DATE: Entered STN: 19920424
Last Updated on STN: 19920424
Entered Medline: 19920408

AB Tumor necrosis factor (TNF) is a cytokine with pleiotropic effects upon cell growth, inflammation and immunologic responsiveness. High-affinity TNF receptors (TNFRs) of 55 and 75 kDa are found in many cell types. Using an Epstein-Barr virus (EBV)-based mammalian expression **library**, we have isolated a clone from human lymphoblastoid transfectants that induces overexpression of the TNFR-encoding gene (TNFR). Transfectants overproducing the TNFR were isolated by multiple rounds of sorting on a **fluorescence**-activated cell sorter using **fluorescent** TNF ligand binding as the selection procedure. Among the sorted transfectants were cells producing approx. 150,000 receptors per cell (Kd of approx. 1 nM). These cells have multiple copies of the TNFR gene present as extrachromosomal plasmids. These cells also overproduced the mRNA for TNFR. Low-Mr EBV episomes were isolated from these overproducing cells and used to transform Escherichia coli. One of the colonies isolated contained a plasmid encoding a portion of the noncoding region of the TNFR gene. Transfection of human lymphoblastoid cells with this DNA gave rise to high-level production of TNFR. **Fluorescent** TNF bound to these transfectants is fully and specifically displaced by an excess of TNF. The rescued clone contains approx. 10 kb of human genomic DNA including the 3'-untranslated region of TNFR and several Alu sequences; apparently during the selection procedure in human cells, recombination occurred to

rescue a portion of the TNFR gene. Transient transfection was used to narrow down the region responsible for TNFR induction to 5.2 kb. The mechanism by which this clone induces TNFR expression has not been determined.

L5 ANSWER 52 OF 66 MEDLINE
ACCESSION NUMBER: 92245414 MEDLINE
DOCUMENT NUMBER: 92245414 PubMed ID: 1315456
TITLE: Cloning muscle isoforms of neural cell adhesion molecule using an episomal shuttle vector.
AUTHOR: Pan L C; Margolskee R F; Blau H M
CORPORATE SOURCE: Pharmacology Department, Stanford University School of Medicine, California 94305.
CONTRACT NUMBER: HD18179 (NICHD)
HD20203 (NICHD)
SOURCE: SOMATIC CELL AND MOLECULAR GENETICS, (1992 Mar) 18 (2) 163-77.
Journal code: UY2; 8403568. ISSN: 0740-7750.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199206
ENTRY DATE: Entered STN: 19920619
Last Updated on STN: 19920619
Entered Medline: 19920602

AB Three distinct transcripts encoding two phosphatidylinositol (PI) linked isoforms of the neural cell adhesion molecule (NCAM) are induced during the differentiation of C2C12 myoblasts into myotubes. Corresponding NCAM clones were isolated from a mouse muscle cDNA **library** made in an Epstein-Barr virus shuttle vector that replicates extrachromosomally in human cells. Following transfection with the **library**, human cells expressing mouse NCAM were enriched using the **fluorescence** -activated cell sorter. Episomal NCAM clones recovered from sorted cells contain an 18-bp insert between exons 12 and 13. Two other NCAM cDNAs encode identical polypeptides containing a 108-bp insert homologous to the complete MSD1 domain, but differ in their 3' untranslated regions. Induction of MSD1-containing transcripts in advance of myotube formation suggests that muscle-specific NCAMs contribute to myogenesis from the earliest stages of differentiation. Moreover, our studies demonstrate the feasibility of cloning tissue-specific molecules by transfection and expression of cDNA libraries in episomal vectors.

L5 ANSWER 53 OF 66 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 89275866 EMBASE
DOCUMENT NUMBER: 1989275866
TITLE: Human chromosome-specific DNA libraries: Construction and purity analysis.
AUTHOR: Fuscoe J.C.; McNinch J.S.; Collins C.C.; Van Dilla M.A.
CORPORATE SOURCE: Biomedical Sciences Division, Lawrence Livermore National Laboratory, Livermore, CA 94550, United States
SOURCE: Cytogenetics and Cell Genetics, (1989) 50/4 (211-215).
ISSN: 0301-0171 CODEN: CGCGBR
COUNTRY: Switzerland
DOCUMENT TYPE: Journal
FILE SEGMENT: 022 Human Genetics
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB We report the construction of eight human chromosome-specific DNA libraries. Metaphase chromosomes were purified by flow-sorting, and the extracted DNA was cleaved with HindIII before cloning into .lambda.Charon 21A. There is now a complete digest HindIII **library** containing greater than five chromosome equivalents for each human chromosome. These are available to the scientific community through the American Type Culture Collection in Rockville, MD. The amount of hamster DNA in libraries in which the chromosome was sorted from human x hamster hybrid cells was estimated by species-specific hybridization. It ranged from 5% to 39%. The sorted chromosomes were examined by **fluorescence** in situ hybridization with species-specific DNA, and the main source of the hamster DNA contamination was found to be intact hamster chromosomes. In addition, we examined a chromosome 21 **library**, LL21NS02, for clones that fail to grow on the rec+ host LE392. Less than 0.6% of the recombinant phage exhibited the rec+-inhibited phenotype.

L5 ANSWER 54 OF 66 MEDLINE DUPLICATE 14
ACCESSION NUMBER: 88320490 MEDLINE
DOCUMENT NUMBER: 88320490 PubMed ID: 3413114
TITLE: A highly conserved repetitive DNA sequence, (TTAGGG)n, present at the telomeres of human chromosomes.
AUTHOR: Moyzis R K; Buckingham J M; Cram L S; Dani M; Deaven L L; Jones M D; Meyne J; Ratliff R L; Wu J R
CORPORATE SOURCE: Genetics Group, LS-3, Los Alamos National Laboratory, University of California 87545.
CONTRACT NUMBER: RR01315 (NCRR)
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1988 Sep) 85 (18) 6622-6.
Journal code: PV3; 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-J04078; GENBANK-JO4078; GENBANK-M19946
ENTRY MONTH: 198810
ENTRY DATE: Entered STN: 19900308
Last Updated on STN: 19970203
Entered Medline: 19881013

AB A highly conserved repetitive DNA sequence, (TTAGGG)n, has been isolated from a human recombinant repetitive DNA **library**. Quantitative hybridization to chromosomes sorted by **flow cytometry** indicates that comparable amounts of this sequence are present on each human chromosome. Both **fluorescent** in situ hybridization and BAL-31 nuclease digestion experiments reveal major clusters of this sequence at the telomeres of all human chromosomes. The evolutionary

conservation of this DNA sequence, its terminal chromosomal location in a variety of higher eukaryotes (regardless of chromosome number or chromosome length), and its similarity to functional telomeres isolated from lower eukaryotes suggest that this sequence is a functional human telomere.

L5 ANSWER 55 OF 66 MEDLINE
ACCESSION NUMBER: 87260984 MEDLINE
DOCUMENT NUMBER: 87260984 PubMed ID: 2955415
TITLE: Carcinoembryonic antigen family: expression in a mouse L-cell transfectant and characterization of a partial cDNA in bacteriophage lambda gt11.
AUTHOR: Kamarck M E; Elting J J; Hart J T; Goebel S J; Rae P M; Nothdurft M A; Nedwin J J; Barnett T R
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1987 Aug) 84 (15) 5350-4. Journal code: PV3; 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-M17191
ENTRY MONTH: 198708
ENTRY DATE: Entered STN: 19900305
Last Updated on STN: 19900305
Entered Medline: 19870826

AB Genomic DNA and mRNA from the adenocarcinoma cell line LoVo were used to generate L-cell transfectants and a bacteriophage lambda gt11 cDNA clone that express epitopes of carcinoembryonic antigen (CEA). Primary and secondary L-cell transfectants expressing CEA were selected with a **fluorescence**-activated cell sorter (FACS). These transfectants, including some clones that were selected for high-level CEA expression by multiple rounds of FACS sorting, express a surface protein of 150 kDa that reacts with all anti-CEA antibodies tested. In parallel, a cDNA **library** of LoVo poly(A)+ RNA was constructed in lambda gt11 and fusion proteins were screened with polyclonal antisera against CEA. One positive clone, lambda cLV7, was identified that hybridized specifically to transfectant DNA. The nucleic acid sequence of the cDNA insert (cLV7) contained two regions of extensive internal homology, with greater than 70% identity at the amino acid level. cLV7 hybridized to three mRNA species of LoVo cells and to a predominant mRNA of the CEA-expressing transfectants. Hybridization of cLV7 to restriction endonuclease-digested genomic DNA of colon carcinoma cells, normal human cells, and human-mouse somatic cell hybrids revealed the presence of multiple hybridizing bands, one of which was present in transfectant cells. These CEA-related sequences are not rearranged in tumors and, by somatic cell hybrid analysis, were mapped to human chromosome 19.

L5 ANSWER 56 OF 66 MEDLINE DUPLICATE 15
ACCESSION NUMBER: 88054312 MEDLINE
DOCUMENT NUMBER: 88054312 PubMed ID: 3677921
TITLE: Human chromosome-specific repetitive DNA sequences: novel markers for genetic analysis.
AUTHOR: Moyzis R K; Albright K L; Bartholdi M F; Cram L S; Deaven L L; Hildebrand C E; Joste N E; Longmire J L; Meyne J; Schwarzacher-Robinson T
CORPORATE SOURCE: Genetics Group, Los Alamos National Laboratory, University of California, NM 87545.
CONTRACT NUMBER: RR01315 (NCRR)
SOURCE: CHROMOSOMA, (1987) 95 (6) 375-86. Journal code: D7A; 2985138R. ISSN: 0009-5915.
PUB. COUNTRY: GERMANY, WEST: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-M20262; GENBANK-M20263
ENTRY MONTH: 198712
ENTRY DATE: Entered STN: 19900305
Last Updated on STN: 19970203
Entered Medline: 19871231

AB Two recombinant DNA clones that are localized to single human chromosomes were isolated from a human repetitive DNA **library**. Clone pHuR 98, a variant satellite 3 sequence, specifically hybridizes to chromosome position 9qh. Clone pHuR 195, a variant satellite 2 sequence, specifically hybridizes to chromosome position 16qh. These locations were determined by **fluorescent** in situ hybridization to metaphase chromosomes, and confirmed by DNA hybridizations to human chromosomes sorted by **flow cytometry**. Pulsed field gel electrophoresis analysis indicated that both sequences exist in the genome as large DNA blocks. In situ hybridization to intact interphase nuclei showed a well-defined, localized organization for both DNA sequences. The ability to tag specific human autosomal chromosomes, both at metaphase and in interphase nuclei, allows novel molecular cytogenetic analyses in numerous basic research and clinical studies.

L5 ANSWER 57 OF 66 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 86209738 EMBASE
DOCUMENT NUMBER: 1986209738
TITLE: Cloning of DNA libraries from mouse Y chromosomes purified by **flow cytometry**.
AUTHOR: Baron B.; Metezeau P.; Hatat D.; et al.
CORPORATE SOURCE: Unite d'Immunogenetique Humaine (Inserm U.276), Institut Pasteur, 75724 Paris Cedex 15, France
SOURCE: Somatic Cell and Molecular Genetics, (1986) 12/3 (289-295). CODEN: SCMGDN
COUNTRY: United States
DOCUMENT TYPE: Journal
FILE SEGMENT: 022 Human Genetics
LANGUAGE: English

AB To purify mouse Y chromosomes by **flow cytometry**, a male cell line containing the Robertsonian translocation Rb(9.19)163H has been established by SV40 transformation. Flow karyotypes obtained from these cells exhibit a well-isolated peak of **fluorescence** corresponding to the single Y chromosome, clearly distinct from that of

chromosome 19. From this peak, 650,000 chromosomes were sorted, and two restriction fragment libraries were constructed from the DNA of the sorted chromosomes. The characterization of several Y-specific fragments has shown that the Y DNA was enriched at least 36-fold. Furthermore, given that there are likely homologies between the X and Y chromosomes, we can assume that this calculated value of the purification factor is an underestimation and that the Y DNA was more highly purified by flow sorting.

L5 ANSWER 58 OF 66 MEDLINE DUPLICATE 16
ACCESSION NUMBER: 87132326 MEDLINE
DOCUMENT NUMBER: 87132326 PubMed ID: 3816174
TITLE: SCAN: a program **library** for high resolution slit-scan analysis of chromosomes in **flow cytometry**.
AUTHOR: Weier H U; Eisert W G
SOURCE: COMPUTER METHODS AND PROGRAMS IN BIOMEDICINE, (1986 Dec) 23 (3) 269-76.
Journal code: DOH; 8506513. ISSN: 0169-2607.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198703
ENTRY DATE: Entered STN: 19900303
Last Updated on STN: 19900303
Entered Medline: 19870330
AB Computer programs for high resolution slit-scan chromosome analysis in **flow cytometry** are introduced. A modular program **library**, SCAN, contains programs for single and dual parameter data acquisition, correction of recorded profiles and computation of histograms of various parameters. Using a minicomputer system, data acquisition is programmed in assembler to realize high input rates and real-time histogram calculation. Software for the processing of recorded profiles has been written in FORTRAN and allows extensions or alterations for different objectives. A sample run recording bicolor **fluorescence** profiles from metaphase chromosomes demonstrates the main features of the software.

L5 ANSWER 59 OF 66 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 85063328 EMBASE
DOCUMENT NUMBER: 1985063328
TITLE: Flow-cytometric analysis and sorting of human metaphase chromosomes. A new field of research.
AUTHOR: Tranebjaerg L.; Vissing H.; Mikkelsen M.
CORPORATE SOURCE: Kromosomlaboratoriet, John F. Kennedy Instituttet, DK-2600 Glostrup, Denmark
SOURCE: Ugeskrift for Laeger, (1985) 147/4 (239-242).
CODEN: UGLAAD
COUNTRY: Denmark
DOCUMENT TYPE: Journal
FILE SEGMENT: 022 Human Genetics
LANGUAGE: Danish
SUMMARY LANGUAGE: English
AB Flow-cytometric characterisation of suspended metaphase chromosomes obtained from various cultured human cells is feasible for karyotyping purposes. Certain advantages of **flow-cytometry** applied as a diagnostic tool in cytogenetics are presented: 1) Quick, reproducible analysis of a large number of cells (106-107). 2) The resulting flow-histogram is constant for each individual patient. On the other hand, some limitations of diagnostic **flow cytometry** exist: 1) Consideration of the patient (a large volume of blood is necessary to obtain a sufficient number of lymphocytes). 2) Pronounced inter-individual variation in the flow-histograms obtained from normal persons is the consequence of heterochromatic polymorphism for a number of chromosomes. 3) Precise statement of specific chromosomes to specific peaks of the **fluorescence**-histograms presumes visual (in **fluorescence** microscope) analysis of individual chromosome fractions. **Fluorescence**-activated chromosome sorting (FACS) is a feasible method of isolating specific chromosomes in sufficient numbers for biochemical studies of DNA. By means of FACS is it possible: 1) to establish libraries of DNA-sequences from specific chromosomes (gene-**library**) as already reported concerning chromosome X, Y, 21 and 22; 2) to map genes to individual chromosomes or specific parts of chromosomes; and 3) to isolate otherwise unidentified marker chromosomes from tumours in order to investigate whether DNA-sequence homology with identified chromosomes can be found.

L5 ANSWER 60 OF 66 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 85170360 EMBASE
DOCUMENT NUMBER: 1985170360
TITLE: Human chromosome analysis by **flow cytometry**.
AUTHOR: Young B.D.
CORPORATE SOURCE: Medical Oncology Laboratory, Imperial Cancer Research Fund, St. Bartholomew's Hospital, London EC1, United Kingdom
SOURCE: Annals of the New York Academy of Sciences, (1985) VOL. 450/- (11-23).
CODEN: ANYAA
COUNTRY: United States
DOCUMENT TYPE: Journal
FILE SEGMENT: 022 Human Genetics
LANGUAGE: English
AB There have been many attempts to fractionate metaphase chromosomes by centrifugation, countercurrent distribution, and lg sedimentation. While such methods can separate chromosomes from the other cellular constituents, and have achieved enrichment of different size classes of chromosomes, the purification of single chromosomes was not possible. Only in recent years has it become possible to fractionate chromosomes with a high degree of resolution. The application of the **fluorescence** -activated cell sorter has allowed the purification of small but useful quantities of individual chromosomes, and therefore has become the method of choice for tackling a number of biological questions. With recombinant DNA techniques, it is now also possible to generate a large number of DNA

probes for a single flow-sorted chromosome and this will prove extremely useful in the linkage analysis of certain genetic disorders.

L5 ANSWER 61 OF 66 MEDLINE
ACCESSION NUMBER: 84181960 MEDLINE
DOCUMENT NUMBER: 84181960 PubMed ID: 6232119
TITLE: Development and use of metaphase chromosome flow-sorting methodology to obtain recombinant phage libraries enriched for parts of the human X chromosome.
AUTHOR: Lalande M; Kunkel L M; Flint A; Latt S A
CONTRACT NUMBER: GM21121/33579 (NIGMS)
HD04807/18658 (NICHD)
SOURCE: CYTOMETRY, (1984 Mar) 5 (2) 101-7.
Journal code: D92; 8102328. ISSN: 0196-4763.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198406
ENTRY DATE: Entered STN: 19900319
Last Updated on STN: 19970203
Entered Medline: 19840619

AB Metaphase chromosomes isolated from human lymphoblastoid cell lines containing structurally abnormal X chromosomes have been stained with the bisbenzimidazole dye Hoechst 33258 and analyzed on a FACS II flow system equipped with a 5-W all-lines argon ion laser. The chromosomal **fluorescence** has been highly resolved at flow rates of 1,000-3,000 chromosomes per second. With the goal of obtaining recombinant DNA libraries from parts of the human X chromosome, **fluorescence** populations enriched for a dicentric X (Xpter- greater than Xq24::Xq24-greater than Xpter) chromosome and an isochromosome of the long arm of the X [i(Xq)] have been identified. The dicentric X chromosome has been resolved as a discrete peak in the **fluorescence** flow histogram. In contrast, the **fluorescence** intensity of the isochromosome is indistinguishable from that of chromosomes 3 and 4. Recombinant DNA libraries from the flow-sorted chromosomes have been constructed in the lambda phage, Charon 21A, and consist of 1.6 X 10(5) and 0.7 X 10(5) plaque-forming units in the case of the dicentric X and the isochromosome, respectively. Ninety percent of the phage in both recombinant libraries contain inserts which hybridize to highly repetitive human DNA sequences. The recombinant phage **library** from the flow-sorted dicentric X chromosome, which could be assigned to a discrete **fluorescence** peak, has been further characterized and shows at least a tenfold enrichment for X chromosome-specific DNA sequences as determined by Southern blot hybridization of cloned fragments.

L5 ANSWER 62 OF 66 MEDLINE
ACCESSION NUMBER: 83247418 MEDLINE
DOCUMENT NUMBER: 83247418 PubMed ID: 6575396
TITLE: Isolation of amplified DNA sequences from IMR-32 human neuroblastoma cells: facilitation by **fluorescence**-activated flow sorting of metaphase chromosomes.
AUTHOR: Kanda N; Schreck R; Alt F; Bruns G; Baltimore D; Latt S
CONTRACT NUMBER: CA-14501 (NCI)
CA-26717 (NCI)
HD-04807 (NICHD)
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1983 Jul) 80 (13) 4069-73.
Journal code: PV3; 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198308
ENTRY DATE: Entered STN: 19900319
Last Updated on STN: 19970203
Entered Medline: 19830811

AB Human neuroblastoma IMR-32 cells have large homogeneously staining regions (HSRs), primarily in the short arms of chromosome 1. We have constructed a recombinant phage **library** that is enriched for DNA present in the HSR of this chromosome by using **fluorescence**-activated flow sorting for initial chromosome purification. Eleven distinct cloned DNA segments were identified that showed significantly greater hybridization to IMR-32 genomic DNA, detected by Southern blotting, than to normal human genomic DNA. These sequences have also been localized to the HSR of chromosome 1 by in situ hybridization. Based on an approximate 50-fold sequence amplification for each cloned segment and a total HSR size of 150,000 kilobases, the amplified unit in the HSR is estimated to be 3,000 kilobases. Sequences homologous to all cloned HSR DNA segments were mapped to human chromosome 2 by using human-mouse hybrid cells. Further work using in situ hybridization demonstrated that cloned HSR segments were localized in the short arm of chromosome 2 in both normal and IMR-32 cells. Thus, the amplification of these sequences in IMR-32 cells may have involved transposition from chromosome 2 to chromosome I.

L5 ANSWER 63 OF 66 MEDLINE
ACCESSION NUMBER: 84003819 MEDLINE
DOCUMENT NUMBER: 84003819 PubMed ID: 6193931
TITLE: Purifying human Y chromosomes by **flow cytometry** and sorting.
AUTHOR: Fantes J A; Green D K; Cooke H J
SOURCE: CYTOMETRY, (1983 Jul) 4 (1) 88-91.
Journal code: D92; 8102328. ISSN: 0196-4763.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198311
ENTRY DATE: Entered STN: 19900319
Last Updated on STN: 19900319
Entered Medline: 19831123

AB A method of producing an enriched sample of human Y chromosomes from peripheral blood lymphocytes is described. Metaphase chromosomes were prepared from peripheral blood lymphocytes donated by 17 normal male

individuals. A suspension of chromosomes in a polyamine buffer was produced from each sample, stained with the **fluorescent** dye Hoechst 33258, and passed through a flow cytometer and sorter. Following analysis of the 17 **fluorescence** distributions, a single donor was found giving a separate peak corresponding to the Y chromosome. Seventy percent of the chromosomes sorted from this peak were identified as Y chromosomes. Batches of a million Y chromosomes were produced from each of several 40 ml donations of peripheral blood. These were assessed for the amount of Y DNA present and used to construct a DNA **library**.

L5 ANSWER 64 OF 66 MEDLINE DUPLICATE 17
ACCESSION NUMBER: 82222258 MEDLINE
DOCUMENT NUMBER: 82222258 PubMed ID: 6953442
TITLE: Construction and characterization of genomic libraries from specific human chromosomes.
AUTHOR: Krumlauf R; Jeanpierre M; Young B D
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1982 May) 79 (9) 2971-5. Journal code: PV3; 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198208
ENTRY DATE: Entered STN: 19900317
Last Updated on STN: 19900317
Entered Medline: 19820814

AB Highly purified fractions of human chromosomes 21 and 22 were isolated from a suspension of metaphase chromosomes stained with ethidium bromide by using a **fluorescence**-activated cell sorter (FACS II). Two recombinant DNA libraries, representing chromosomes 21 and 22, were constructed by complete digestion of DNA from these fractions with EcoRI and insertion into the vector lambda gtWES lambda B. Twenty clones selected at random from the chromosome 22 **library** hybridized to EcoRI-digested human DNA, and five of these clones hybridized to single bands identical in size to the phage inserts. These five single-copy sequences and a clone coding for an 8S RNA isolated by screening the chromosome 22 **library** for expressed sequences were characterized in detail. Hybridization of all six clones to a panel of sorted chromosomes and hybrid cell lines confirmed the assignment of the sequences to chromosome 22. The sequences were localized to regions of chromosome 22 by hybridization to translocated chromosomes sorted from a cell line having a balanced translocation t(17;22)(p13;q11) and to hybrid cell lines containing the various portions of another translocation t(X;22)(q13;q112). Five clones reside on the long arm of chromosome 22 between q112 and pter, while one clone and an 18S rRNA gene isolated from the chromosome 22 **library** reside pter and g112. The construction of chromosome-specific libraries by this method has the advantage of being direct and applicable to nearly all human chromosomes and will be important in molecular analysis of human genetic diseases.

L5 ANSWER 65 OF 66 MEDLINE
ACCESSION NUMBER: 82174330 MEDLINE
DOCUMENT NUMBER: 82174330 PubMed ID: 6461845
TITLE: Regional localization on the human X of DNA segments cloned from flow sorted chromosomes.
AUTHOR: Kunkel L M; Tantravahi U; Eisenhard M; Latt S A
CONTRACT NUMBER: GM21121 (NIGMS)
HD 40807 (NICHD)
SOURCE: NUCLEIC ACIDS RESEARCH, (1982 Mar 11) 10 (5) 1557-78. Journal code: O8L; 0411011. ISSN: 0305-1048.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198206
ENTRY DATE: Entered STN: 19900317
Last Updated on STN: 19970203
Entered Medline: 19820614

AB **Fluorescence** activated sorting of chromosomes from 49,XXXXY human lymphoblasts has been used to obtain DNA enriched for the human X. This DNA was cloned in lambda phage Charon 21A to obtain a **library** of approximately 60,000 pfu. Phage inserts free of human highly repeated DNA sequences are localized to different regions of the human X by two independent hybridization analyses. The first utilized comparative hybridization to rodent-human hybrid cell DNA samples containing all or known portions of the human X, while the second was based on hybridization dosage to DNA samples from human cell lines differing in the number of X chromosomes or X chromosome segments. Of five unique sequence inserts tested, three were X chromosome specific and were localized to regions Xpter leads to Xcen, Xql leads to Xq22 and Xq24 leads to Xqter, respectively. The **library** presented here represents a highly enriched source of human X chromosome-specific DNA sequences.

L5 ANSWER 66 OF 66 MEDLINE DUPLICATE 18
ACCESSION NUMBER: 82013644 MEDLINE
DOCUMENT NUMBER: 82013644 PubMed ID: 6456416
TITLE: Cloning of a representative genomic **library** of the human X chromosome after sorting by **flow cytometry**.
AUTHOR: Davies K E; Young B D; Elles R G; Hill M E; Williamson R
SOURCE: NATURE, (1981 Oct 1) 293 (5831) 374-6. Journal code: NSC; 0410462. ISSN: 0028-0836.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198111
ENTRY DATE: Entered STN: 19900316
Last Updated on STN: 19900316
Entered Medline: 19811122

AB A **library** of 50,000 recombinants representative of the human X chromosome has been constructed. Human X chromosomes were physically

separated using a **fluorescence**-activated cell sorter. The DNA was purified from the chromosomes, digested to completion with the restriction enzyme EcoRI and cloned into the phage lambda gtWES.lambda B. The X-derived nature of the recombinants was confirmed by hybridization to rodent/human cell line DNA containing only the human X chromosome. Such libraries will be particularly useful for the investigation of genetic diseases such as Duchenne muscular dystrophy, where the basic defect has not been elucidated, and of neoplasia, where several specific chromosomal anomalies, particularly for the leukaemias, have been identified.